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THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF CERTAIN CEREAL HAYS AS AFFECTED BY PLANT MATURITY [1]

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INTRODUCTION

The stage of maturity at which cereal plants are cut for hay is an important factor in the chemical composition, palatability, digestibility, aroma, texture, and color of the hay. The study herein reported was made to determine the effect of maturity on the chemical composition of certain cereal plants and to ascertain the apparent digestibility of some of the organic nutrients in these plants. Milk, medium-dough, and fully ripe stages of kernel maturity were selected for the studies in digestion; all stages of the life cycle were included in the chemical analyses.

REVIEW OF LITERATURE

IMMATURE CEREAL PLANTS

Florell and Faulkner (*11*) [3] made daily measurements of wheat plants and observed that wheat culms elongated rapidly during the heading stage, the rate of elongation depending upon the temperature. The elongation of the culms continued as long as blossoming persisted; little or no growth occurred after that stage.

Trowbridge, Haigh, and Moulton (*36*) found that wheat plants take up nitrogen and mineral matter most rapidly in the young stages and at decreasing rates as growth proceeds. The heads gain more rapidly and uniformly in dry matter than any other part of the plant. Nitrogen-free extract is produced and stored at a greater rate than any other constituent, but nitrogen, ash, and ether extract are added in some quantity also. The fiber is practically all formed by the time the blossoms disappear. The wheat stems and leaves contain their maximum dry matter at blossoming time, after which some of it is passed to the heads. Nitrogenous materials and nitrogen-free extract appear to be the constituents which the stalks and leaves yield up to the heads.

MATURE CEREAL PLANTS

Brenchley and Hall (*6*) observed that synthesis of new reserve food almost ceases during the latter part of the life of the wheat plants, and migration of nutrients from stem and leaves is dominant.

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[3] Reference is made by number (italic) to Literature Cited, p. 414.

McLean (*22*), working with barley, found that ash in the leaves and in whole plants tended to decrease, whereas in the heads a rise was followed by a period of uniform content up to the eighteenth day before full ripeness, when a decrease occurred. The protein of the leaves and culms increased to maturity. The heads decreased in percentage of protein until the kernels reached the soft-dough stage, after which a slight increase was noted.

R. W. Thatcher (*33*, *34*, *35*) and Kiesselbach (*17*) working with wheat, and Harlan (*12*) with barley, noticed increases in the percentage of total nitrogen of the kernels with the approach of maturity, though some of the increases were small. LeClerc and Breazeale (*20*) and Kedzie (*16*) reported less nitrogen in grain harvested at maturity than in that harvested 7 days earlier.

Newton and Brown (*24*) observed a reduction in moisture and an increase in sugars during the fall, in hard wheat varieties. Sharp (*28*) found that synthesis of protein ceased when the moisture content of the kernels reached about 35 percent.

Harlan and Pope (*13*) found that an 80-percent moisture content in barley kernels at flowering was followed by a decrease to 42 percent, at which point the deposition of dry matter was interrupted, with a subsequent rapid dessication of the kernels.

Brenchley (*5*) found that as barley plants mature, with the accumulation of inactive substances such as cellulose and lignin, there is relatively less tissue capable of growth. In the barley kernel growth limitation is largely a matter of starch congestion. Starch deposition proceeds until stopped by low moisture, by lack of reserve in the plant, and by tension of the cell walls.

STAGES OF MATURITY

Parkins and his coworkers (*25*) reported that in feeding practice, the milk- or early soft-dough stage is preferred, before the seed drains the leaves and stems of plant food.

Bedford (*3*) cut wheat in the early-milk, late-dough, and ripe stages. Wheat harvested a week early in the dough stage produced as good a yield as that cut at maturity.

Thatcher (*32*) in Ohio concluded that, for cattle or sheep, oats should be cut when the kernels are in the milk- to soft-dough stage. Horses will make good use of oats cut when a little more mature. Barley of bearded varieties should be cut at the milk stage before the beards become tough. Rye is satisfactory if cut not later than the full-bloom stage.

In Ohio (*32*) oat hay weighed 7 percent more than ripe oats and straw combined; barley hay was 1 percent heavier; and rye hay harvested at full bloom was less than the total yield of straw. Barbee (*1*) reported straw and grain yields for Washington oat varieties from which hay yield estimates may be made, which, of course, will apply only to certain farming areas. Schafer, Gaines, and Barbee (*27*) reported yields of Washington barleys.

Briggs, as reported by Crozier (*8*), harvested wheat plants daily with the full-length straw attached, beginning when the grain was in the milk stage and continuing until it was fully ripe. The increase in weight because of translocation of nutrients from the straw was greatest for cuttings made at the milk and dough stages. This conclusion is supported by McDowell (*21*).

Hendry and Woll (*14*) reported yields of 5.95, 8.22, 8.32, and 7.35 tons of air-dry oat hay at the blossom, milk, soft-dough, and ripe stages, respectively.

Taylor and Martin (*31*) pointed out that the straw-grain ratio of cereals is influenced by the length of the stubble. Musgrave (*23*) has correlated straw and grain yields.

DIGESTIBILITY OF CEREAL PLANTS

Parkins and his co-workers (*26*) fed wheat hay to horses, studying full-bloom stage; stages 6, 13, 27, and 34 days after bloom; and fully ripe stages. The dry matter decreased in apparent digestibility, the coefficients for all the intervals declining from 54 to 49. The protein digestibility showed no regular trend, being 60 at full bloom, 53, 20 days after bloom, and 61 at full maturity. The fiber digestibility was higher at bloom and 6 days after bloom than at later stages. Parkins et al. concluded that wheat hay cut at full bloom is more highly digestible than any cut later, and, in general, the digestibility of wheat hay for horses decreases by regular intervals as the period of complete ripeness is approached. Hay cut at full bloom had a 12-percent advantage over that cut a week before ripening. When the cutting was postponed until the dough stage, a reduction in total yield and in quality because of reduced digestibility resulted.

Bartlett (*2*), in studies with sheep, found only small differences in the digestibility of dry matter of oat plants harvested at the bloom, milk, and dough stages. The coefficients of apparent digestibility for protein of 53.5, 58.6, and 44.7 showed a dropping off, at the dough stage. For crude fiber—and in the same order—the figures were 59.9, 50.3, and 49.4, and for nitrogen-free extract, 51.2 55.0, and 59.1.

EXPERIMENTAL METHODS AND MATERIALS

Cross-bred range lambs, confined in specially constructed metabolism crates, were used in the digestion trials. The sampling and analytical methods and other details have been fully discussed by the author (*29*) in a previous publication.

The cereals used in the studies were: Three varieties of wheat, Pacific Bluestem, Fortyfold, and Albit; one variety of oats, Markton; one variety of barley, Horsford; and one variety of rye, Rosen. For comparative purposes analyses were also made of Grimm alfalfa and common white sweetclover.

Pacific Bluestem wheat (*Triticum aestivium L.*) is a spring variety having a long spike and a white kernel. Fortyfold is a winter variety with a long spike and white kernel, and is subject to smut infection. It has a purple straw and shatters readily. Albit, a club wheat (*T. compactum Host*) is a winter variety with a stiff straw. It is awnless, has a white kernel, and is resistant to some forms of smut.

The Markton variety of oats (*Avena sativa L.*) has a large panicle and a distinct pubescence just above the last node. It is immune to covered smut and yields well. Horsford barley (*Hordeum vulgare L.* var. *horsfordianum*) is a six-rowed, covered, hooded, spring variety. Rosen rye (*Secale cereale L.*) is a winter variety, sown for early spring pasture.

Grimm alfalfa (*Medicago sativa L.*) is quite winter-hardy and of fairly fine texture, with a good proportion of leaves.

Common white blossom sweetclover (*Melilotus alba* Desv.) is a vigorously growing variety used for pasture, silage, and hay.

All of the forages were grown on Palouse silt loam, a typical soil type for the area in which the experimental plots were located. The forage samples were dried on canvas to avoid losses from shattering.

All samples of forage used in the growth studies were cut at the ground line; those used in the digestion studies were cut with a binder, leaving an average stubble of 4 inches.

VEGETATIVE STAGES IN RELATION TO CHEMICAL COMPOSITION

Chemical analyses were made of the cereal and legume plants at different stages in their life cycle. The data are summarized in table 1. The rate of growth in inches as related to age in days is given for intervals ranging from 3 to 14 days or more, covering the complete life cycle.

A close similarity is observed between the cereal and legume plants in the younger stages. On a dry-matter basis young cereal plants are high-protein feeds, in this respect resembling alfalfa and sweetclover. As the cereal plants matured, their cereal characteristics asserted themselves. The fiber and nitrogen-free extract increased, and fat, protein, and ash decreased. This result is in accord with that of Swanson (*30, pp. 22-23*). Crowther and Ruston (*7*), and Kirk, Davidson, and Hamilton (*18*).

Almost invariably the dry matter of the cereal plants decreased in both calcium and phosphorus as the plants matured. This result is also shown by Knowles and Watkin (*19*).

TABLE 1.—*Chemical composition of the dry matter of cereal and legume plants in relation to stages of maturity*

Cereal hay and number of sample used	Date harvested	Height	Stage	Age	Water	Composition on a water-free basis						
						Ash	Crude protein (N×6.25)	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus
Pacific Bluestem wheat:		*Inches*		*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	June 8, 1933	29–30	Boot	64	80.97	9.19	14.50	27.16	45.42	3.73	0.24	0.42
2	July 5, 1933	33–36	One-half to two-thirds headed	71	72.34	7.99	12.15	25.52	51.23	3.11	.19	.31
3	July 12, 1933	42–44	Dough	78	62.53	6.94	9.79	27.64	52.99	2.64	.17	.29
Fortyfold wheat: 1	July 13, 1933	38–40	do		55.43	5.89	8.33	21.32	62.11	2.35	.14	.26
Albit wheat:												
1	June 14, 1933	18–20	Heads out of boot	243	77.39	8.49	10.57	25.07	52.53	2.87	.24	.18
2	June 21, 1933	42	Well headed	250	68.21	7.45	9.09	28.46	52.33	2.64	.20	.15
3	June 28, 1933	45	Some anthers	257	67.62	7.87	9.14	28.50	52.25	2.22	.20	.16
4	July 5, 1933	45	Kernels in water stage	264	60.12	6.67	7.14	25.40	58.71	2.06	.15	.15
5	July 8, 1933		Late milk	267	[1] 15.73	6.42	9.08	23.72	58.78	1.94	.14	.16
6	July 14, 1933		Medium dough	273	[1] 16.93	5.97	9.36	23.39	59.14	2.07	.16	.17
7	July 25, 1933		Dead ripe	284	[1] 16.57	6.71	8.05	25.96	57.60	1.63	.17	.19
1	Mar. 20, 1934	6	Very young	156	82.12	11.28	28.88	14.20	39.43	6.21	.42	.69
2	Apr. 3, 1934	10–12	Young	171	83.00	11.91	22.68	15.58	44.30	5.53	.43	.60
3	Apr. 19, 1934	16–18	do	186	81.84	11.92	13.46	20.57	50.11	3.94	.35	.42
4	May 1, 1934	24		198	80.77	12.73	9.51	25.69	49.48	2.59	.23	.43
5	May 14, 1934	34		211	75.51	10.17	5.45	25.21	56.83	2.34	.28	.41
6	May 28, 1934	48	Heads in boot	225	73.27	8.72	4.85	30.48	53.80	2.15	.26	.27
7	June 8, 1934	54		236	70.80	9.16	4.50	32.41	52.09	1.84	.18	.23
8	June 15, 1934	57–60		243	64.69	8.39	4.58	31.12	54.59	1.32	.14	.23
9	June 20, 1934	58–63	Milk	248	64.05	7.69	4.43	31.57	54.37	1.94	.13	.23
10	June 25, 1934	58–63	Early dough	253	57.29	8.27	3.86	28.18	57.89	1.80	.12	.21
11	July 2, 1934	58–63	Medium dough	260								
12	July 23, 1934	58–63	Dead ripe	281								
Rosen rye:												
1	July 15, 1933	40–50	Dough		57.68	5.14	10.60	24.87	57.17	2.22	.28	.30
1	Mar. 20, 1934	12		155	80.69	11.99	28.01	17.34	36.24	6.42	.50	.59
2	Apr. 3, 1934	12–14		169	79.82	11.92	17.20	18.89	47.46	4.53	.44	.50
3	Apr. 19, 1934	26–28		185	82.46	8.38	11.79	23.52	52.44	3.87	.41	.43
4	May 1, 1934	44	Half boot	197	79.74	8.94	11.44	29.19	47.08	3.35	.36	.45
5	May 14, 1934	58	Full bloom	210	76.90	7.56	11.80	35.44	42.24	2.96	.44	.30
6	July 11, 1934	72	Dead ripe	240	68.20	6.14	5.41	32.20	54.29	1.96	.23	.21
Markton oats:												
1	June 14, 1933	12	Young	65	85.33	10.97	31.69	20.18	30.89	6.27	.41	.40
2	June 21, 1933	22	do	72	79.69	9.55	15.16	20.28	50.73	4.28	.31	.52
3	June 28, 1933	28	Heads out of sheath	79	70.20	7.95	12.75	20.20	55.98	3.12	.31	.29
4	July 5, 1933		Kernels one-fourth formed	86	72.66	8.32	10.22	26.78	51.36	3.32	.27	.26

[1] Dry.

TABLE 1.—*Chemical composition of the dry matter of cereal and legume plants in relation to stages of maturity*—Continued

Cereal hay and number of sample used	Date harvested	Height	Stage	Age	Water	Composition on a water-free basis						
						Ash	Crude protein (N×6.25)	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus
Markton oats—Con.		*Inches*		*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
5	July 12, 1933	41–43	Milk	93	65.90	7.45	10.85	28.83	51.82	3.05	.31	.29
6	July 17, 1933		Medium dough	98	[1] 15.47	7.44	9.23	30.44	49.33	3.56	.32	.27
1	May 16, 1934	7–8	Young	34	85.58	11.73	19.96	14.51	50.77	3.03	.55	.77
2	May 28, 1934	12–13	do	43	81.42	9.25	10.86	18.48	57.35	4.06	.42	.35
3	June 8, 1934	21–22	do	59	80.93	8.37	9.07	20.49	59.23	2.84	.38	.33
4	June 15, 1934	27–28	do	66	79.28	7.54	8.34	26.43	54.67	3.02	.31	.33
5	June 25, 1934	36–38	Heads out of boot	74	71.84	6.66	6.92	29.70	53.68	3.04	.30	.26
6	July 2, 1934		Milk	81	72.48	6.81	7.68	29.74	52.88	2.89	.28	.30
Horsford barley:												
1	June 14, 1933	16		55	84.34	9.70	16.98	20.24	48.29	4.79	.33	.46
2	June 21, 1933	26	Bloom on some heads	62	82.36	7.99	17.00	22.78	48.55	3.68	.37	.35
3	June 28, 1933	35	Full bloom	69	79.47	7.21	9.94	28.40	52.16	2.29	.23	.33
4	July 5, 1933	42–43	Kernels water stage	76	74.71	6.88	9.17	27.80	53.86	2.29	.21	.25
5	July 8, 1933		Milk	79	[1] 15.74	7.77	8.38	32.34	49.58	1.93	.26	.31
6	July 14, 1933		Medium dough	85	[1] 14.24	7.20	7.95	24.16	58.36	2.33	.19	.29
7	July 26, 1933		Dead ripe	97	[1] 15.02	7.51	7.29	19.25	64.41	1.54	.14	.30
Sweetclover (white blossom) second year:												
1	Mar. 20, 1934	3–4	Very young		83.88	11.96	29.64	8.62	45.28	4.50	1.73	.59
2	Apr. 3, 1934	6–7	do		84.52	10.90	23.90	9.48	51.14	4.58	2.11	.49
3	Apr. 19, 1934	14	do		86.51	10.66	22.06	12.31	50.47	4.50	1.91	.45
4	May 1, 1934	24	do		87.27	9.80	18.94	14.71	52.18	4.37	1.37	.42
5	May 14, 1934	38	do		85.18	8.29	16.08	27.50	44.67	3.46	1.18	.35
6	May 22, 1934	42–44	Cut for hay in early bud		82.09	5.38	12.71	28.30	50.13	3.48	1.19	.46
Alfalfa (Grimm) second year:												
1	Mar. 20, 1934	6	Very young		79.96	13.46	26.43	15.07	40.57	4.47	1.52	.53
2	Apr. 3, 1934	6–8	Young		80.37	11.64	25.90	16.18	41.38	4.90	1.52	.50
3	Apr. 19, 1934	6–8	do		83.15	11.08	24.74	18.99	40.87	4.90	1.44	.53
4	May 1, 1934	22–24			83.56	9.71	22.20	24.64	40.10	3.35	1.33	.47
5	June 14, 1934	27			76.90	8.22	16.73	29.47	42.00	3.58	1.55	.31
6	June 28, 1934	41	Early bud		76.71	7.51	14.35	33.25	41.83	3.06	1.58	.31
7	July 8, 1934	44–46			77.59	7.80	13.28	34.78	41.63	2.51	1.82	.32
8	July 11, 1934	48–50	One-half bloom (cut for hay)			7.73	13.69	35.64	40.83	2.11	1.77	.32

[1] Dry.

Table 2 shows the weight distribution between heads, stems, and leaves of air-dry samples in stands of wheat, oats, and barley, and indicates how this distribution is affected by maturity. In fully ripe Albit wheat, the heads are approximately 60 percent by weight, the stems only 15 to 20 percent, and the leaves 21 to 23 percent. Berry (*4*) reported similar data for oats.

The dough stage shows only a slightly lower proportion of heads by weight than does the ripe stage of Albit wheat. A great difference in varieties of wheat exists at this stage, the Pacific Bluestem being much leafier than the Albit. Plants of Markton oats, Albit wheat, and Horsford barley at the dough stage are almost equally leafy.

Markton oats show a regular decline in the percentage of leafiness as the plants mature, although the actual weight of leaves may not change. Some of this decrease undoubtedly is attributable to a translocation of food nutrients to the ripening seeds, but some of it is due to a diluting effect of the starch stored in the rapidly developing endosperm. The leaf percentage is irregular in Albit wheat and shows no decrease in Horsford barley past the dough stage. Some of the leaf loss is mechanical, and some is due to a translocation of food nutrients.

The data in table 2 are based on plants cut 4 inches above the ground. Since precautions were taken to prevent losses from shattering when harvesting at ripe stages, the ripe-forage samples studied experimentally are not strictly comparable to field-cured samples.

TABLE 2.—*Weight relationship of leaves, stems, and heads of cereal plants at different stages of maturity*

Stage and plant part	Albit wheat hay			Pacific Bluestem wheat hay		Markton oat hay				Horsford barley hay	
	1933	1934	1935	1933	1935	1932	1933	1934	1935	1933	1935
Milk:	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Leaves	21.92	22.10	.....	.....	.....	26.80	.....	.....	.....	22.79	.....
Stems	33.51	37.30	.....	.....	.....	41.50	.....	.....	.....	36.84	.....
Heads	44.57	40.60	.....	.....	.....	31.70	.....	.....	.....	40.37	.....
Dough:											
Leaves	19.92	.....	.....	26.61	.....	20.90	26.49	.....	.....	20.74	.....
Stems	26.55	.....	.....	40.25	.....	27.80	28.87	.....	.....	29.96	.....
Heads	53.53	.....	.....	33.14	.....	51.30	44.64	.....	.....	49.30	.....
Ripe:											
Leaves	20.20	.....	15.10	.....	17.20	18.60	.....	.....	15.26	20.87	16.64
Stems	22.15	.....	23.20	.....	21.30	26.20	.....	.....	19.32	15.21	17.18
Heads	57.65	.....	61.70	.....	61.50	55.20	.....	.....	65.42	63.92	66.18

The oat heads contained 8.8 percent of chaff and the barley and wheat 8.14 and 23.91 per cent, respectively, when fully ripe.

As subsequent data will show, the physical composition (stem-leaf-head ratio) of the cereal plants is a fairly reliable and practical index of its feeding value. The ratio of stem, leaf, and head in a cereal plant affects both its nutritive value and its chemical composition, and for this reason, such measurements have been made. In comparison with the data of Hill (*15*), and Fagan and Watkin (*10*), the results show that the Palouse section produces cereal plants with a very favorable ratio of seed to stem and leaf.

When cereal hay crops are permitted to mature, they lose nutritive value under field conditions through loss of leaves and shattering of grain. This fact is shown by Parkins and his coworkers (*25*), Doneen (*9*), and Trowbridge, Haigh, and Moulton (*36*).

The composition of field-cured cereal hays is given in table 3. The dry matter in the samples studied varied from 82 to slightly above 90 percent. Horsford barley and Markton oat hays were slightly higher in ash than hay from Albit and Pacific Bluestem wheat. The oat hays were higher in crude protein than either the Albit wheat or the barley hays, and the Pacific Bluestem wheat hay contained least protein. In fiber Pacific Bluestem wheat hay was highest and oats next; not much difference was noted between Albit wheat and Horsford barley. The oat hays were outstandingly higher in fat. As compared with alfalfa hay, all the grain hays were lower in calcium and phosphorus.

It is recognized that the milk, medium-dough, and fully ripe stages of kernel maturity of the various cereal plants do not always represent the same relative maturity of the vegetative parts. For instance, on bottom land, Markton oat hay cured from plants with kernels in the medium-dough stage may still have green leaves and stems, whereas at the same stage on the hilltops, the stems and leaves may be quite bleached and the leaves brittle.

TABLE 3.—*Chemical composition of field-cured cereal hays fed during the digestion experiments*

Cereal hay and year harvested	Stage	Dry matter	Ash	Crude protein (N×6.25)	Carbohydrates		Fat	Calcium	Phosphorus
					Crude fiber	Nitrogen-free extract			
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Albit wheat, 1933	Milk	84.27	5.41	7.66	20.00	49.56	1.64	0.12	0.14
	Dough	83.07	4.96	7.78	19.44	49.17	1.72	.13	.14
	Ripe	83.43	5.60	6.72	21.66	48.09	1.36	.14	.16
Pacific Bluestem wheat, 1933	Dough	82.00	5.97	5.29	27.77	40.93	2.04	.14	.24
Markton oats, 1932	Milk	90.21	6.87	9.32	28.39	42.24	3.38	------	------
	Dough	88.50	6.15	9.04	24.00	45.38	3.93	------	------
	Ripe	90.37	6.21	9.22	24.22	46.90	3.73	------	------
Markton oats, 1933	Dough	84.53	6.29	7.80	25.73	41.70	3.01	.27	.22
Horsford barley, 1933	Milk	84.26	6.55	7.07	27.27	41.74	1.63	.22	.26
	Dough	85.76	6.18	6.82	20.72	50.04	2.00	.16	.25
	Ripe	84.98	6.39	6.20	16.37	54.71	1.31	.12	.25

Table 4 shows the chemical composition on a water-free basis of various cereal plants, their leaves, and their stems taken at stages suitable for hay.

The figures for the whole plants of Albit wheat show that as the kernels developed there was a diluting effect, with a resultant decrease in ash, protein, fiber, and fat. The leaves also decreased in percentage of ash, protein, and fat past the dough stage, although increasing in fiber. The decrease in protein was especially large. Fiber, of course, increased. With the exception of ash, the nutrients of the stems followed a similar trend.

Crude protein, fiber, and fat decreased in Horsford barley plants as they matured. In all cases the nitrogen-free extract was more abundant in the more mature plants.

The leaves of all the cereals studied had higher calcium values than either the whole plants or the stems. In Albit wheat no marked changes were observed in the calcium and phosphorus of the whole plants within the limits of the milk and ripe stages. The leaves

contained 3.4 to 5.7 times as much calcium as phosphorus, and the heads contained more than 3 times as much phosphorus as calcium. Horsford barley leaves contained 1.5 to 1.9 times as much calcium as of phosphorus.

The stems increased in fiber as they matured, although the composition of the entire plant showed a decrease in this nutrient. The diluting influence of the developing nonfibrous endosperm tended to mask the actual fiber increase in the stems.

TABLE 4.—*Chemical composition (on water-free basis) of whole plants, leaves, stems, and heads of various cereal plants, 1933 crop*

Cereal hay, plant part, and stage	Ash	Crude protein (N×6.25)	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus
Albit wheat:							
Whole plants:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Milk	5.80	9.08	23.49	59.80	1.83	0.141	0.152
Dough	6.28	8.81	23.31	59.27	2.30	.154	.165
Ripe	6.15	8.12	21.76	62.23	1.74	.160	.182
Leaves:							
Milk	12.26	7.18	29.39	47.38	3.79	.334	.097
Dough	14.12	6.17	28.87	46.73	4.11	.385	.087
Ripe	13.25	3.83	30.87	48.98	3.07	.364	.064
Stems:							
Milk	2.64	3.78	34.88	57.30	1.40	.097	.065
Dough	3.10	3.80	38.86	53.00	1.24	.128	.106
Ripe	3.57	2.24	40.67	52.65	.87	.127	.106
Heads:							
Milk	4.68	13.10	12.75	68.00	1.47	.087	.249
Dough	4.85	12.72	11.63	69.19	1.61	.079	.249
Ripe	4.04	13.23	9.02	72.43	1.28	.065	.226
Pacific Bluestem wheat:							
Whole plants, dough	7.30	6.56	30.85	53.23	2.06	.118	.289
Leaves, dough	12.32	6.41	31.95	45.25	4.07	.225	.229
Stems, dough	3.49	3.58	39.85	51.50	1.58	.085	.202
Heads, dough	6.08	10.14	19.88	62.07	1.83	.076	.412
Markton oats:							
Whole plants, dough	7.37	10.16	29.36	49.22	3.89	.310	.267
Leaves, dough	11.74	10.20	31.67	41.83	4.56	.645	.204
Stems, dough	6.34	5.80	39.62	46.84	1.31	.129	.194
Heads, dough	4.70	13.76	18.42	57.49	5.63	.249	.368
Horsford barley:							
Whole plants:							
Milk	7.01	10.20	29.79	50.92	2.08	.256	.299
Dough	7.13	8.32	22.64	59.90	2.01	.182	.288
Ripe	7.45	6.95	18.61	65.29	1.70	.139	.288
Leaves:							
Milk	14.04	8.14	28.82	45.09	3.91	.471	.310
Dough	16.77	10.09	26.56	42.73	3.85	.441	.290
Ripe	15.01	8.21	29.81	43.02	3.95	.510	.266
Stems:							
Milk	3.67	3.78	38.92	52.44	1.19	.119	.194
Dough	4.18	3.09	36.46	55.05	1.22	.107	.150
Ripe	6.07	2.18	47.86	43.05	.84	.063	.073
Heads:							
Milk	6.29	13.29	17.13	61.67	1.66	.108	.421
Dough	5.59	10.16	11.84	70.89	1.52	.098	.348
Ripe	4.80	8.97	8.76	76.11	1.35	.086	.388

The cereal plants at the milk stage in all instances were higher in protein and lower in nitrogen-free extract than were the corresponding plants when ripe. Horsford barley at the milk stage contained more protein than did Albit wheat, but the protein decreased more rapidly. Ripe Albit wheat plants contained more protein than did ripe Horsford barley plants.

In order to bring out the relative importance of stems, leaves, and heads in any given stand of cereals, a percentage distribution table was computed. Table 5 shows the distribution of protein and other nutrients in stems, leaves, and heads. Of the total protein in ripe Albit wheat plants 85.59 percent was found in the heads. Similarly,

75 percent of the phosphorus was found in the heads. These values are relative, and in Albit wheat and Horsford barley show further the changes in nutrient distribution among stems, leaves, and heads as affected by maturity.

Table 5 also shows the close association of fiber with stems, of total ash with leaves, of crude protein with heads, of nitrogen-free extract with heads, of calcium with leaves, and of phosphorus with heads.

TABLE 5.—*Distribution of nutrients in stems, leaves, and heads of various cereal plants, assuming each nutrient as 100 percent*

Cereal hay, stage, and plant part	Weight distribution	Water	Crude ash	Crude protein (N × 6.25)	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus
Albit wheat:									
Milk:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stems	33.51	30.61	15.68	14.69	49.16	32.41	24.17	21.42	14.28
Leaves	21.92	20.33	47.61	18.18	27.07	17.50	42.31	50.00	14.28
Heads	44.57	49.06	36.71	67.13	23.77	50.09	33.52	28.58	71.44
Dough:									
Stems	26.55	17.57	13.60	11.72	47.17	24.18	16.94	21.43	17.66
Leaves	19.92	14.58	45.94	14.08	26.10	15.94	41.53	50.00	11.76
Heads	53.53	67.85	40.46	74.20	26.73	59.88	41.53	28.57	70.58
Ripe:									
Stems	22.15	19.03	13.70	5.69	44.35	18.61	12.41	23.07	12.50
Leaves	20.20	19.77	46.30	8.72	30.44	15.67	40.00	53.86	12.50
Heads	57.65	61.20	40.00	85.59	25.21	65.72	47.59	23.07	75.00
Pacific Bluestem wheat:									
Dough:									
Stems	40.25	36.44	21.15	22.35	51.77	39.16	27.64	27.27	29.63
Leaves	26.61	25.92	49.03	26.33	27.33	22.62	46.54	54.55	25.93
Heads	33.14	37.64	29.82	51.32	20.90	38.22	25.82	18.18	44.44
Markton oats:									
Dough:									
Stems	28.87	28.53	26.03	16.17	40.80	26.92	9.23	10.34	20.00
Leaves	26.49	25.38	44.26	25.69	29.97	22.11	29.55	55.17	20.00
Heads	44.64	46.09	29.71	58.14	29.23	50.97	61.22	34.49	60.00
Horsford barley:									
Milk:									
Stems	36.84	38.02	18.96	16.16	51.43	35.36	22.04	22.22	23.33
Leaves	22.79	21.07	45.37	21.68	23.74	18.97	44.62	55.56	23.33
Heads	40.37	40.91	35.67	62.16	24.83	45.67	33.34	22.22	53.34
Dough:									
Stems	29.96	26.96	16.88	11.62	49.29	27.61	19.20	20.00	15.38
Leaves	20.74	19.63	46.61	26.22	24.77	14.77	41.81	53.33	23.08
Heads	49.30	53.41	36.51	62.16	25.94	57.62	38.99	26.67	61.54
Ripe:									
Stems	15.21	10.36	13.19	4.41	38.63	10.44	7.02	6.25	3.45
Leaves	20.87	18.77	44.23	22.24	32.52	14.12	45.61	62.50	17.24
Heads	63.92	70.87	42.58	73.35	28.85	75.44	47.37	31.25	79.31

It is of interest to note that in a stand of Albit wheat when the kernels are at the dough stage, approximately half the ash and half the calcium of the crop are in the leaves, almost 75 percent of the protein and 60 percent of the starch are in the heads, and 47 percent of the fiber is in the stems.

Ripe Albit wheat hay contained 86 percent of its protein and 66 percent of its nitrogen-free extract in the heads. Only 6 percent of the total crop protein was in the stems at maturity. The stems have a diluting effect on the nutritive value of the grain hay, especially in ripe wheat.

In marked contrast to wheat, Markton oats at the dough stage showed only 58 percent of the plant protein in the kernels as against 16 percent in the stems. Kernels of Albit wheat at the same stage contain 74 and stems 12 percent. The heads of oats contained slightly more than half of the plant nitrogen-free extract, whereas the stems had about 27 percent. Albit wheat contained 60 percent nitrogen-

free extract in the heads and 24 percent in the stems. Stems and leaves of the oat plant at the dough stage contain a larger share of the total plant protein, fiber, and nitrogen-free extract than do leaves and stems of Albit wheat. In the process of ripening, the Albit wheat plants translocate to the heads more of the organic nutrients than do Markton oat plants at similar stages.

Horsford barley at the medium-dough stage resembled Albit wheat in respect to nutrient distribution among leaves, stems, and heads. The protein contained in the leaves, however, was slightly higher in barley.

In general, half or more of the calcium of a stand of wheat was in the leaves, the other half being distributed between the stems and heads. Phosphorus, on the other hand, was to a larger extent confined to the heads.

DIGESTION STUDIES

The average coefficients of apparent digestibility obtained with lambs for the various organic nutrients of the different samples of cereal hay studied are shown in table 6. The same six lambs were used in all the 1933 studies. As Albit wheat developed from the milk to the dough stage, the digestibility of the various nutrients did not change much, but from the dough to the ripe stage a decrease occurred in the digestibility of protein, and an increase in the digestibility of dry matter and fiber.

The dry matter of Markton oat hay was more digestible in the mature plants than at the milk stage. The crude protein increased in digestibility as the plants matured. The crude fiber was least digestible in the oat plant at the dough stage, but it was about equally digestible at the milk and ripe stages. The nitrogen-free extract was well digested at the milk and dough stages and showed a rise in the ripe stage. The same was true of crude fat.

The same general trend was noted for Horsford barley hay as for Markton oat hay. The rise in the digestibility of dry matter was more pronounced, however. There was a distinct rise in the digestibility of protein and nitrogen-free extract as the plants matured; the reverse was true of fiber.

TABLE 6.—*Coefficients of apparent digestibility of nutrients of various cereal hays*

[Each value is an average of 6 coefficients and is followed by its standard deviation] [1]

Cereal hay and stage	Year harvested	Dry matter	Protein (N×6.25)	Crude fiber	Nitrogen-free extract	Crude fat
Albit wheat:						
Milk	1933	50.8±5.2	58.1±5.1	33.7±4.1	62.1±6.1	39.7±5.2
Dough	1933	52.4±2.3	58.8±1.5	34.5±2.4	63.6±2.4	40.9±7.9
Ripe	1933	56.9±3.5	54.9±2.2	47.1±2.9	65.4±2.0	43.0±2.1
Pacific Bluestem wheat, dough	1933	51.1±6.3	44.0±7.5	49.6±6.1	58.4±5.5	46.4±6.1
Markton oats:						
Milk	1931	54.8±.44	63.2±.55	52.5±.83	56.7±.49	71.5±1.22
Dough	1931	51.7±.82	64.2±.82	42.5±1.42	56.0±.54	71.2±.94
Ripe	1931	59.7±1.51	69.5±1.02	51.5±1.50	62.7±.78	74.5±1.36
Dough	1933	57.3±3.6	66.7±2.7	54.5±4.2	59.7±3.7	57.7±4.8
Horsford barley:						
Milk	1933	50.9±4.0	49.1±6.6	52.1±4.6	57.4±3.3	43.7±5.0
Dough	1933	59.9±2.0	54.7±3.2	47.4±3.6	70.8±1.5	57.5±3.6
Ripe	1933	61.8±3.7	54.9±4.8	40.9±6.6	75.2±2.6	45.8±7.1

[1] The formula used in computing the standard deviation was $\sigma = \pm\sqrt{\frac{\Sigma\, d^2}{(n-1)}}$.

The digestible nutrients contained in the various forages were computed by using the coefficients determined with lambs. Each coefficient is an average of 6 independent determinations. For cereal hays, as table 7 shows, do not contain a large quantity of digestible crude protein. Albit wheat and Horsford barley hays had a higher content of total digestible nutrients with advancing maturity. Markton oat hay also was richer in digestible nutrients when the plants were ripe than when they were in the milk stage. These results were secured with samples of hay in which no shattering occurred. The rather lower value obtained at the dough stage seems difficult to explain.

Oat hay had a narrower nutritive ratio than hay of either wheat or barley. Horsford barley hay, although lower than Albit wheat hay in digestible crude protein at all stages, was higher in total digestible nutrients at the dough and ripe stages. Pacific Bluestem wheat hay was much lower in both digestible crude protein and total digestible nutrients than any of the other cereals studied.

The composition of the feces of the lambs used in the experiments is shown in table 8 and their weight in table 9.

TABLE 7.—*Effect of stage of plant maturity on the digestible nutrient content of cereal hays*

Cereal hay and stage	Year harvested	Digestible nutrients						
		Total dry matter	Crude protein (N× 6.25)	Carbohydrates	Fat	Total digestible nutrients	Nutritive ratio 1 to—	Rating on basis of total digestible nutrients
Albit wheat:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		
Milk	1933	84.27	4.45	37.52	0.65	43.43	8.76	98.4
Dough	1933	83.07	4.57	37.98	.70	44.13	8.66	100.0
Ripe	1933	83.43	3.69	41.64	.58	46.63	11.64	105.7
Pacific Bluestem wheat, dough	1933	82.00	2.33	37.67	.95	42.14	17.09	95.5
Markton oats:								
Milk	1931	90.21	5.89	38.85	2.42	50.18	7.52	.....
Dough	1931	88.50	5.80	35.61	2.80	47.71	7.23	.....
Ripe	1931	90.37	6.41	41.93	2.78	54.56	7.53	.....
Dough	1933	84.53	5.20	38.91	1.74	48.02	8.24	108.8
Horsford barley:								
Milk	1933	84.26	3.47	38.17	.71	43.24	11.46	98.0
Dough	1933	85.76	3.73	45.25	1.15	51.57	12.83	116.8
Ripe	1933	84.98	3.40	47.84	.60	52.59	14.47	119.1

TABLE 8.—*Composition of feces of lambs fed cereal hays*

[Each figure is an average for 6 lambs]

Cereal hay and stage	Water	Ash	Crude protein (N×6.25)	Carbohydrates		Fat
				Crude fiber	Nitrogen-free extract	
Albit wheat:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Milk	55.94	5.47	3.42	14.15	20.08	1.06
Dough	56.42	5.23	3.52	14.06	19.65	1.11
Ripe	54.85	5.43	3.76	14.32	20.66	.97
Pacific Bluestem wheat, dough	61.74	4.77	2.83	13.38	16.32	1.04
Markton oats, dough	61.92	3.87	2.77	12.37	17.72	1.34
Horsford barley:						
Milk	63.87	4.85	3.15	11.64	15.68	.79
Dough	61.74	5.47	3.44	12.15	16.24	.95
Ripe	52.35	8.40	4.16	14.18	19.87	1.04

TABLE 9.—*Average weight of lambs fed various cereal hays during 10-day periods*

[Each figure is an average for 6 lambs]

Cereal hay and stage	Initial	Final	Average	Cereal hay and stage	Initial	Final	Average
	Kilograms	*Kilograms*	*Kilograms*		*Kilograms*	*Kilograms*	*Kilograms*
Albit wheat:				Markton oats, dough	29.26	30.72	29.99
Milk	29.37	30.39	29.88	Horsford barley:			
Dough	28.99	29.75	29.37	Milk	30.24	29.90	30.07
Ripe	29.33	29.90	29.61	Dough	29.03	30.73	29.88
Pacific Bluestem wheat, dough	30.46	31.00	30.73	Ripe	31.48	31.22	31.35

The palatability of cereal hays appears to be related to (1) curing conditions, which affect aroma and freedom from molds and dust; (2) variety; some varieties are sweeter than others since they vary in percentage of the different sugars, proteins, etc. (this condition may be affected by soil type and climate); (3) texture, some being more fibrous than others even at corresponding stages of maturity; (4) maturity, for incident to ripening there is a translocation of plant food materials from leaves and stems to the heads and possibly some to the roots; (5) rate of seeding, since this affects physical texture.

The form and manner in which hay is handled is very important in determining its feeding value. When fed bundle hay, animals select the heads and the finer portions, thereby consuming a forage rich in digestible nutrients. The same hay, if so ripe that the kernels have been shattered or consumed by birds, mice, or other animals, may approximate straw in feeding value.

Table 10 shows in what quantities the nutrients of the cereal hays were consumed by lambs per 100 pounds of body weight. Milk-stage Albit wheat hay supplied the most protein. Medium-dough-stage Markton oat hay in the quantities consumed was the leading source of calcium, and was also relatively high as a source of phosphorus.

TABLE 10.—*Relative quantities of feed and nutrients consumed, computed to 100 pounds (45.36 kg) body weight as consumed by lambs (per head daily) full-fed various cereal hays*

Cereal hay and stage	Average weight per lamb based on 6 head	Feed and nutrients consumed per head daily, computed to 100 pounds body weight of lamb							
		Feed as fed	Dry matter	Crude protein (N×6.25)	Carbohydrates		Fat	Calcium	Phosphorus
					Crude fiber	Nitrogen-free extract			
	Kilograms	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Albit wheat:									
Milk	29.8	1,188.0	1,001.0	91.0	237.6	588.7	19.48	1.44	1.61
Dough	29.3	1,098.4	912.4	85.4	213.5	540.1	18.89	1.43	1.58
Ripe	29.5	1,066.2	889.5	71.6	230.9	512.7	14.50	1.47	1.68
Pacific Bluestem wheat, dough	30.8	1,092.3	895.7	57.8	303.3	447.1	22.28	1.49	2.64
Markton oats, dough	30.0	1,147.8	970.2	89.5	295.3	478.6	34.55	3.06	2.58
Horsford barley:									
Milk	30.1	935.6	788.3	66.1	255.1	390.5	15.25	2.04	2.43
Dough	29.9	1,310.5	1,116.1	88.7	269.6	651.3	26.03	2.08	3.26
Ripe	31.3	1,031.1	876.2	63.9	168.8	564.1	13.51	1.26	2.59

With Albit wheat at the dough stage again taken as a base, a palatability index was computed on the basis of dry matter consumed; the data are contained in table 11. Since the lambs varied in weight, the consumption by lambs was computed to a 100-pound base. The data show the following order of preference on the basis of dry matter consumed: (1) Horsford barley (dough stage); (2) Albit wheat hay (milk stage); (3) Markton oat hay (dough stage); (4) Albit wheat hay (dough stage); (5) Pacific Bluestem wheat hay (dough stage); (6) Albit (ripe); (7) Horsford barley (ripe); (8) Horsford barley hay (milk-stage).

TABLE 11.—*Palatability index based on Albit wheat hay cut at the dough stage*

[Consumed daily per 100 pounds body weight as a percentage of that eaten when Albit wheat medium-dough stage, was fed]

Cereal hay and stage	Rating on basis, feed fed	Rating on basis, dry matter consumed	Cereal hay and stage	Rating on basis, feed fed	Rating on basis, dry matter consumed
Albit wheat:	*Percent*	*Percent*		*Percent*	*Percent*
Milk	108.1	109.7	Markton oat dough	104.4	106.3
Dough	100.0	100.0	Horsford barley:		
Ripe	97.1	97.4	Milk	85.1	86.4
Pacific Bluestem wheat, dough	99.4	98.1	Dough	118.4	122.3
			Ripe	93.8	96.0

SUMMARY AND CONCLUSIONS

Chemical analyses were made of several cereal plants at different stages of maturity and of two legumes. The proportion by weight and the chemical composition of stems, leaves, and heads of the more mature stages were determined. Experiments with lambs were conducted to determine the digestibility of Albit wheat, Markton oats, and Horsford barley in the milk, medium-dough, and ripe stages, and of Pacific Bluestem wheat in the dough stage.

In the young stages the composition of the dry matter of the cereals was found to be practically identical with that of alfalfa and sweetclover. The young plants contained 28 to 31 percent protein on the water-free basis and resembled protein concentrates. In all grain hays studied the young plants on a dry-matter basis were richer in calcium and phosphorus than were the mature ones.

Albit wheat plants cut with a 4-inch stubble when mature, contained on an air-dry basis 60 percent heads, 15 to 20 percent leaves, and 22 to 23 percent stems. The heads of oats, barley, and wheat contained 9 percent, 8 percent, and 24 percent chaff, respectively.

The stem-leaf-head ratio of cereal plants is a fairly reliable index of their nutritive values.

As Albit wheat plants developed, the ash, protein, fiber, and fat decreased on a percentage basis. The fiber in the stems increased. The same was true of Horsford barley. The leaves of all the cereals studied contained more calcium and phosphorus than either the whole plants or the stems.

In all instances cereal plants in the milk stage were higher in protein and lower in nitrogen-free extract than were the ripe plants. Pacific Bluestem wheat plants were the lowest and Markton oat plants the highest in protein. In the cereals there was a close association of fiber with stems; of total ash and calcium with leaves; and of phosphorus, crude protein, and nitrogen-free extract with heads.

In a normal stand of Albit wheat with the kernels at the dough stage, half the ash and half the calcium of the crop were in the leaves, almost 75 percent of the protein and 60 percent of the starch were in the heads, and 47 percent of the fiber was in the stems. In fully ripe Albit wheat plants only 6 percent of the total crop protein was in the stems.

Markton oat kernels in the dough stage contained 58 percent of the plant protein, and the stems contained 16 percent.

Horsford barley resembled Albit wheat very closely in this respect.

Seventy-five percent of the total phosphorus at the ripe stage was found in the heads of wheat. Half or more of the calcium in a stand of wheat was in the leaves and the other half equally distributed between the stems and the heads.

As Albit wheat plants matured from the milk stage to full ripeness, the digestibility of the fiber, nitrogen-free extract, and fat increased. The crude protein showed no significant change in digestibility from the milk to the dough stage, but it did decrease from the dough to the ripe stage. The dry matter of Markton oat hay was more digestible in mature plants than at the milk stage. The crude protein increased in digestibility as the plants matured, whereas in wheat it decreased.

Albit wheat and Horsford barley hays showed a higher content of digestibility nutrients as the plants progressed from the milk to the ripe stages. Oat hay had a narrower ratio than either wheat or barley hay. Horsford barley hat at the dough stage, although lower in digestible crude protein, was higher in total digestible nutrients than Albit wheat hay at the same stage. Of the cereals studies, Bluestem wheat hay contained the least digestible protein and the smallest amount of total digestible nutrients.

A palatability index shows the following order of preference by lambs, starting with the most palatable hays: (1) Horsford barley at the dough stage, (2) Albit at the milk stage, (3) Markton oat at the dough stage, (4) Albit at the dough stage, (5) Pacific Bluestem wheat at the dough stage, (6) Albit when ripe, (7) ripe Horsford, and (8) milk-stage Horsford.

With dough-stage Albit considered as 100 percent, the following ratings of total digestible nutrients were obtained for the grain hays studied: Ripe Horsford 119.1, medium-dough Horsford 116.8, medium-dough Markton 108.8, ripe Albit 105.7, milk-stage Albit 98.4, and medium-dough Pacific Bluestem 95.5. These are, of course, arbitrary ratings on the basis of the total digestible nutrient content only and as determined with lambs.

These studies seem to indicate that, in practice, the medium-dough stage of plant maturity is the most desirable for Albit wheat, Horsford barley, and Markton oats. Any benefits resulting from increased digestibility past this stage would seem to be more than offset by shattering of the kernels, leaf loss, leaching, and a general deterioration of the plant structure.

Young cereals afford a high protein pasture, and, with the exception of calcium and phosphorus, are almost identical with legume pasture in chemical composition.

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ANALYSIS OF VARIATION IN THE SEXUAL CYCLE AND SOME OF ITS COMPONENT PHASES, WITH SPECIAL REFERENCE TO CATTLE [1]

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INTRODUCTION

Normally there is a periodicity of the reproductive processes of female mammals, and this more or less regular recurrence of sexual phenomena is primarily under hormonic control. It has been shown that the rhythm of the ovarian processes can be disturbed experimentally. It has also been shown that some animals exhibit marked variation in the length of the cycle associated with concurrent physiological disturbances, both functional and pathological. But, in addition to these variations there is also considerable variation which has not been explained on the basis of the above factors.

An attempt has been made in this study of the sexual cycle and its component phases to isolate that part of their variation which is associated with determinable factors.

DATA STUDIED

The records on dairy cattle were taken by the herdsman (a university graduate) of the Pabst Farms, Oconomowoc, Wis. These cattle were Holstein-Friesians and were under uniform management and feeding conditions throughout the 10 successive years covered by these records. The records were kept with unusual accuracy, and the cattle were observed daily. Ovarian changes as shown by rectal palpation, the flow of blood or mucus from the vulva, infections, etc., were recorded. The records on the oestrous cycles of swine were taken, under the direction of Dr. F. F. McKenzie, at the Missouri Agricultural Experiment Station, by a trained investigator who recorded the lengths of the period in hours. In the test for oestrus a vasectomized boar was used. The published records utilized were, as far as possible, accurate records of normal individuals.

SEXUAL CYCLES

GENERAL VARIATION

Examination of the literature makes it evident that the scarcity of accurate records has left the general impression that the length of the sexual cycle is relatively invariable in normal individuals of most species. A study of the more accurate data shows to what a surprising degree this supposition is in error.

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[2] The authors wish to express appreciation to Dr. L. J. Cole, at whose suggestion this work was initiated and carried out; to the management of the Pabst Farms for allowing free access to their records; and to Dr. F. F. McKenzie for the loan of the swine records of the Missouri Agricultural Experiment Station.

Table 1 shows the frequency distribution of the oestrous cycles for the cattle. The same is shown in more detail in figure 1. The total frequency, the mode, the mean and its standard error,[3] and the standard deviation are given in the table.

TABLE 1.—*Oestrous-cycle lengths in clinically normal cattle* [1]

Length (days)	Number	Percentage of total
4–16	34	4.9
17–27	416	60.3
28–33	34	4.9
34 or more	206	29.9
Total	690	100.0

[1] Summary: Total cycles, 690; mode, 21; mean, 32.1±0.8; standard deviation, 20.5. Cycles within the 4- to 33-day range, 484; mode, 21; mean, 21.4±0.2; standard deviation, 4.4.

The distribution of the oestrous-cycle lengths of the clinically normal cattle (as defined below) shows a much wider variation than has been reported before, but the distribution is quite similar in form to that of other data within the range of variation given by other authors.

The variation in the intervals between heats which may be attributed to careless observations is slight. Extremely short and "silent" heat periods are probably responsible for what might be termed "observational error." The multimodality is, in all probability, largely due to these two factors, i. e., failure in observing oestrus when of very short duration or of subthreshold expression.

Table 2 presents the statistics of sexual-cycle lengths of several other species. It is to be noted that the oestrous cycles of cattle used in this study showed significantly greater variation than the sexual cycles observed in other species.

TABLE 2.—*Statistics of sexual-cycle length of species other than cattle* [1]

Species	Reference	Cycles	Mode	Mean	Standard deviation
		Number	*Days*	*Days*	*Days*
Sheep	(*12*) [2]	65	16	16.57±0.10	0.77
Do	(*41*)	590	16	16.58± .03	.72
Do	(*34*)	226	17	17.04± .05	.74
Swine	(*3*)	66	21	21.36± .22	1.77
Do	(*20*)	71	21	20.99± .30	2.54
Horse	(*35*)	594	21	22.79± .12	4.44
Man	(*3, 11, 17, 18, 19, 21, 33*)	2,323	27	27.94± .10	4.73
Baboon	(*46*)	72	.....	31.40± .62	5.24
Monkey	(*8, 15*)	623	28	28.34± .27	6.72

[1] Most of these statistics and those of other tables were calculated from the original data as reported by the investigators referred to.

[2] Reference is made by number (italic) to Literature Cited, p. 433.

[3] MCKENZIE, F. F. Unpublished data on swine at the Missouri Agricultural Experiment Station.

CLINICAL ABNORMALITY

Deviation from the generally accepted length of the sexual cycle is commonly thought to be associated with definite abnormalities, functional or pathological. As a rule, these associated conditions are discussed in general terms, and the limits of the variations in the cycle

[3] The standard error, rather than the probable error, is reported in all cases.

length indicative of them are not specified. It is well known that cystic follicles and persistent corpora lutea are common causes of markedly disturbed periodicity in the sexual cycle.

In order to determine whether variability in cycle length is associated with subsequent clinical abnormality, the records of the cattle were divided, on the basis of the herdsman's observations, into two groups, "clinically normal" and "clinically abnormal." The former included those showing no cystic follicles, retained corpora lutea, or other deviations from the normal; the latter, those which had a record of cystic follicles or persistent corpora lutea in at least one calving interval, but were normal in other respects. The oestrous cycles in both these groups were again divided into two classes; one, "non-copulatory", in which the oestrous cycles were not preceded by

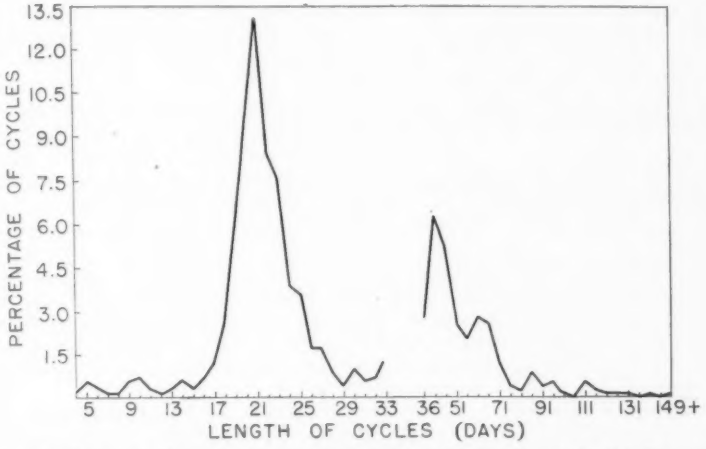

FIGURE 1.—Frequency polygon showing variation in length of the oestrous cycle in clinically normal cattle. The 4- to 33-day range is plotted by 1-day intervals; thereafter the points are plotted on the mid-points of 5-day classes.

service; the other, "copulatory", in which at the beginning of the cycle there was a potentially fertile service (infertile mating with a male of good general fertility).

The cycles in the calving intervals prior to the occurrence of clinical abnormality, in cows which eventually become at least temporarily abnormal, were analyzed for indications of abnormality which they might show. Cycles reported as less than 4 days in length are possibly due to continuous oestrus and are therefore omitted. Those of more than 33 days are thrown into one class (table 3) as being abnormally long on the basis of the normal distribution curve and not likely to yield more information by finer classification. This class is probably in large part due to those factors mentioned above as "observational errors." Of the remainder (those of 4 to 33 days), the distribution curve shows the range of greatest concentration to be from 17 to 27 days. This range includes 62.2 percent of all cycles and 86.8 percent of the cycles less than 34 days in length.

TABLE 3.—*Comparison of noncopulatory oestrous cycles of clinically normal and clinically abnormal cattle* [1]

Length of cycles (days)	Clinically normal		Preceding clinical abnormality	
	Number	*Percent*	*Number*	*Percent*
4–16	28	6.0	12	11.3
17–27	289	62.2	43	40.6
28–33	16	3.4	7	6.6
34 or more	132	28.4	44	41.5
Total	465	100.0	106	100.00

[1] Summary: Total cycles, χ^2, 877.68; P, <0.01. Cycles within the 4- to 33-day range, χ^2, 10.01; P, <0.01.

The test of the difference between the early cycles of cows which became cystic or had retained corpora lutea in later calving intervals, and those of cows which were clinically normal throughout life, was made by means of the χ^2 test of independence as given by Fisher (*10*). In this case (for the range 4–33 days) the difference between the means is not statistically significant[4], but the differences between the distributions tested by χ^2 are highly significant. The reason for the nonsignificant mean difference is that the cycle lengths in the calving intervals preceding the clinical abnormality are concentrated at the two extremes, thus balancing each other, to the extent of making the mean of this group almost identical with the mean of the clinically normal group.

Among the clinically abnormal cows there were 10 which had 5 or more noncopulatory cycles preceding the calving interval in which the abnormality occurred. None of the 10 had as many as 62 percent of these cycles in the 17- to 27-day range, whereas 52 percent of 42 normal cows had at least 62 percent of their 5 or more cycles lying in a similar range.

These facts suggest that extreme variation in the length of the oestrous cycles of a cow is an indication of ovarian abnormality. Presumably, there is disturbed periodicity in the ovarian rhythm before the derangements can be recognized by means of rectal palpation of the ovaries. Irregularity in cycle length may therefore be taken as warning of impending breeding difficulty.

INFERTILE SERVICE

The marked effect of service (copulation) on ovarian behavior is well known in certain animals. In the ferret, cat, and rabbit it serves as a stimulus to ovulation. Furthermore, in those animals, as well as in the rat and mouse, an infertile copulation is succeeded by pseudopregnancy.

The present data afford opportunity for the study of the effect of unsuccessful service by a presumably fertile bull on the length of the oestrous cycle (table 4). Only clinically normal cows were used in testing the significance of the difference between the noncopulatory and copulatory oestrous cycles. Over the entire range and even over the restricted range (4–33 days) the copulatory cycles are significantly longer, on an average, than the noncopulatory. Similarly, the χ^2 test

[4] The word "significant" is used throughout in a statistical sense. If differences of the size given would occur less than 5 percent of the time by chance alone they are arbitrarily considered significant; if less than 1 percent of the time, highly significant.

based either on the tabular classification or on the reduced range points to a highly significant difference between the two distributions. Hammond's data (*13*) do not show increased cycle length when the cycle is preceded by mating with a vasectomized bull. In McKenzie's swine data [5] 8 cycles preceded by service with a potentially fertile male were significantly longer and more variable than the 45 cycles preceded by service with a vasectomized male (mean difference 4.43 ± 1.77 days.) Similar observations on sheep were reported by Terrill (*41*).

TABLE 4.—*Comparison of copulatory and noncopulatory oestrous cycles in clinically normal cattle* [1]

Length of cycle (days)	Noncopulatory		Copulatory	
	Number	*Percent*	*Number*	*Percent*
4–16	28	6.0	6	2.7
17–27	289	62.2	127	56.4
28–33	16	3.4	18	8.0
34 or more	132	28.4	74	32.9
Total	465	100.0	225	100.0

[1] Summary: Total cycles, mean for noncopulatory 30.8 ± 0.9 and for copulatory 34.9 ± 1.5, the mean difference being 4.1 ± 1.8, P being 0.02, χ^2 being 11.5, and $P < 0.01$. Cycles within the 4- to 33-day range, mean for noncopulatory 21.2 ± 0.2 and for copulatory 22.1 ± 0.4, the mean difference being 0.9 ± 0.4, P being 0.04, χ^2 being 10.3, and $P < 0.01$.

These records on cattle, sheep, and swine point to greater average length of copulatory cycles (cycles preceded by potentially fertile mating) as compared with cycles following definitely sterile service (mating with a vasectomized male) or no mating at all. These differences must be ascribed to a temporary reproductive disturbance at the time of, or subsequent to, service. Such disturbance might result in failure either in fertilization or implantation or in early resorption of the embryo. Without more evidence than is at present available it is not possible to decide which of the alternatives is correct.

INDIVIDUALITY

Some investigators have pointed to the marked individual regularity of the sexual cycles; others have stressed their distinct irregularity, but no one has given a definite measure of this individual "repeatability" tendency. By analysis of variance a definite measure of the repetition of characteristic sexual cycle lengths per individual can be made. Only clinically normal cows are considered in the following discussion, and copulatory and noncopulatory cycles are treated separately.

In the first two analyses given in table 5 the entire and 4- to 33-day ranges of noncopulatory oestrous-cycle lengths of the cows were used. These wide distributions do not show any significantly inherent periodicity of oestrus. When, however, the central class (17–27 days) of cycles is analyzed it is found that, on an average, each animal has a very definite individuality in cycle length. The noncopulatory cycles show a modified squared correlation ratio [6] (η^2) of 0.41 for the restricted range (17–27 days). This figure indicates that 41 percent of the observed variance would disappear if all the oestrous cycles were those of one individual rather than many individuals; in other

[5] MCKENZIE, F. F. Unpublished data on swine at the Missouri Agricultural Experiment Station.

[6] This constant differs from the more commonly known correlation ratio in that it allows for the loss of degrees of freedom incurred by dividing the data into arrays and calculating the variations within each such array.

words, if "individual" is "held constant", the total mean square (4.4) is reduced 41 percent (to 2.6). This "repeatability" tendency is masked by more influential factors when the range is not restricted to this class of greatest concentration of cycle lengths. Unidentified abnormality and observational error are probably the main factors in this masking effect.

TABLE 5.—*Analysis of variance in oestrous-cycle lengths clinically normal cattle*

Type of cycle	Range	Source of variation	Degrees of freedom	Sum of squares	Mean square	*P*	η^2
	Days						
	(1)	Individual	73	29,817	408.4	>0.05	0.02
		Remainder	391	138,739	354.8		
		Total	464	168,556	363.3		
Noncopulatory	4–33	Individual	65	1,412	21.7	>0.5	.02
		Remainder	262	5,084	19.4		
		Total	327	6,496	19.9		
	17–27	Individual	60	648	10.8	<.01	.41
		Remainder	216	557	2.6		
		Total	276	1,205	4.4		
Copulatory	17–27	Individual	26	194	7.5	<.01	.19
		Remainder	70	277	4.0		
		Total	96	471	4.9		

[1] Entire.

TABLE 6.—*Individuality of sexual-cycle length in cattle, sheep, swine, horse, monkey, baboon, and man*

Species	Reference	Range	Cycles	Individuals	*P*	η^2
		Days	*Number*	*Number*		
Cattle	(*13*)	(1)	59	15	<0.01	0.58
Sheep	(*34*)	(1)	226	24	<.01	.20
Do	(*12*)	(1)	62	14	>.05	.09
Swine	(2)	(1)	36	15	≒.04	.37
Horse	(*35*)	(1)	38	10	≒.02	.28
Monkey	(*6*)	<50	109	10	[3] ≒.01	.....
Do	(*15*)	<50	514	32	<.01	.06
Baboon	(*16*)	(1)	71	9	>.05	.10
Man	(*5, 11, 17, 18, 19*)	(1)	1,493	88	<.01	.03
Do	(*5, 11, 17, 18, 19*)	19–36	1,406	86	<.01	.43

[1] Entire.
[2] McKENZIE, F. F. See footnote 5, p. 421.
[3] In this group there is greater similarity between the mean cycle lengths of different individuals than between cycle lengths of a single individual. These are cycles of young postpuberal monkeys, which, as is pointed out later, are known to have more variable cycle lengths than mature individuals.

Table 6 summarizes the results obtained by a similar analysis of published records for a number of species. Hammond's records of heifers, (*13*), reported to the accuracy of an hour and with a range of 17½ to 24 days, give additional evidence of inherency of oestrous periodicity in cattle. The analysis of Quinlan and Maré's (*34*) and Grant's (*12*) records for sheep, the Missouri records for swine, and Satô and Hoshi's (*35*) for horses, brings out the same tendency in these species. Likewise in the menstrual cycles of man, monkey, and baboon there is distinct evidence of individuality. Detailed analyses of these are given by Chapman.[7]

[7] For detailed tables of all the data discussed in this paper, reference is made to the following: CHAPMAN, A. B. ANALYSIS OF VARIATION IN THE SEXUAL CYCLE AND SOME OF ITS COMPONENT PHASES Unpublished manuscript on file in the Univ. Wis. library.

This measure of individuality of sexual cycles includes, in addition to the genetic, all other effects on the cycles of an individual which tend to make them more similar to each other than to the cycles of other individuals. It is impossible to say definitely how much of the variation in the sexual-cycle lengths is a permanent or genetic characteristic and how much is due to uniformity of other influences acting on the individual.

AGE

Long and Evans (*24*) and Parkes (*31*) have shown that the earlier cycles of the rat and mouse are longer than those of the more mature animal, whereas Stockard and Papanicolaou (*38*) state that the length of the cycle in guinea pigs tends to increase with age. Hammond (*13*) reports an indication of increased cycle length with age (as judged by the teeth) in cows. Krallinger (*20*) points out a similar relationship in swine. McKenzie and Phillips (*26*), Grant (*12*), and Terrill (*41*) state that no age effect was noticeable in sheep. Satô and Hoshi (*35*) made similar observations on horses. Engle and Shelesnyak (*9*), reporting on the length of the menstrual cycle in man, and Corner (*6*) and Hartman (*15*), on that in monkeys, point to a decrease in variability as menstrual life increases.

In order to study the association between age and cycle length in cattle, the noncopulatory oestrous cycles, i. e., those not preceded by service, were arrayed by calving interval and analyzed for calving interval effect within the restricted range. All calving intervals over the fifth were classed in one group. There was a definite increase in the means from the first to the sixth-and-over calving interval (table 7). This age trend was shown to be significant by analysis of variance. Likewise, comparison of the heifers and cows reported by Hammond indicates an increase in the length of cycle with increasing age, though his numbers are insufficient to establish a significant difference. Five percent of the variance in both sets of data is associated with calving interval or age variation.

TABLE 7.—*Association between age and length of non-copulatory oestrous cycles in clinically normal cattle (17 to 27-day range)*

Calving interval no.	Cycles	Mean length of cycles	Standard deviation	Calving interval no.	Cycles	Mean length of cycles	Standard deviation
	Number	*Days*	*Days*		*Number*	*Days*	*Days*
1	22	20.4	2.1	5	13	22.4	2.2
2	103	21.6	2.1	6 and over	39	22.7	2.1
3	57	21.6	2.0				
4	26	21.9	2.1	Total or average	260	21.7	2.1

The standard deviation of the oestrous-cycle lengths for each calving interval is given in table 7. It is obvious that there is no significant change in variability associated with age changes on the basis of this classification. Comparison of the oestrous cycles in the first two calving intervals with those in all other calving intervals does not show any significant differences between the standard deviations in the two groups, regardless of the ranges used.

In the swine data no difference in mean or variation associated with age was found.

The records for man, summarized in table 8, show a highly significant inverse correlation between age and cycle length and also a significant decrease in variation with increasing age.

TABLE 8.—*Correlation between length of menstrual cycle, variation of cycle, and age of individual in man (11, 17, 18, 19, 21)*

Age (years)	Cycles	Mean length of cycles	Standard deviation
	Number	*Days*	*Days*
17–20	344	28.9±0.42	7.8
21–30	658	28.0± .16	4.0
31–45	408	27.5± .14	2.8
Total or average	1,410	28.1± .13	5.0

SEASON

The restricted breeding season of many animals and the seasonal variation in fertility of others which breed throughout the year testify to the important role that season often plays in reproductive processes.

Ovarian periodicity, insofar as it is marked by oestrus, is one of the functions which is often temporarily suspended at certain times of the year. Less noticeable seasonal effects than complete inhibition also occur. Lee (*22*) has shown that cold tends to lengthen the oestrous cycle of rats. Similarly, in the guinea pig, Stockard and Papanicolaou (*38*) found a slightly longer cycle in winter. Duncan (*8*) states that the menstrual cycle of women in Lapland and Greenland is longer than in hot countries, and Wallace (*42*) gives figures to show that oestrus recurs at shorter intervals in summer than in winter in cows. According to Marshall (*27*) the length of the dioestrous cycle in sheep is reduced when they are taken from highland to lowland. Satô and Hoshi (*35*) point to a gradual shortening of the oestrous cycle in the horse from March to July.

The observations of other authors differ from the above reports of longer cycles in colder weather. Hammond (*13*) reports a longer oestrous cycle in the middle of summer than in winter or spring in cows; Engle and Shelesnyak (*9*) state that long menstrual intervals in puberal girls are most frequently initiated in July, that a low percentage of menarches and a high percentage of temporary amenorrheas occur during the summer months; and Hartman (*15*) records inhibition of ovulation, greater irregularity of menstrual cycles, and increase in amenorrhea in the summer in monkeys.

Analysis of the copulatory and noncopulatory oestrous cycles in the dairy cattle data showed no variation that could be attributed to seasonal changes, regardless of the range considered. Hammond's records, although indicative of longer cycles for the heifers from June to November, do not show a statistically significant effect of season.

In Quinlan and Maré's sheep records (*34*) no evidence of seasonal change is found in the oestrous cycle variation. In Grant's data (*12*), as noted by him, there is, however, a distinct trend to longer oestrous cycles as the breeding season advances. This increase from month to month accounts for 37 percent of the variation in the oestrous cycle length of this group of sheep.

PERIOD FROM PARTURITION TO FIRST SUBSEQUENT OESTRUS

GENERAL VARIATION

Rabbits, rats, guinea pigs, and mice usually come into oestrus a few hours post partum. Rats and mice, however, do not resume the periodicity of oestrus when suckling a normal sized litter. The mare has been reported by Satô and Hoshi (*35*) as coming in heat 3 to 100 days after parturition, but 92 percent of the cases fell between the limits of 5 and 12 days, and shorter intervals occurred in older animals. The sow is reported by Struve (*40*) to come in heat 4 to 9 days post partum. Marshall (*28*), however, believes that 5 weeks is the most frequent interval. Quinlan and Maré's records (*34*) for merino sheep give the time of ovulation as from 10 to 15 days post partum, but they state that heat is expressed at this time only when the ewe is not suckling a lamb. Weber (*43*) gives 3 to 7 weeks for this period in the cow. Hammond (*13*) states the opinion of stockmen to be that 3 to

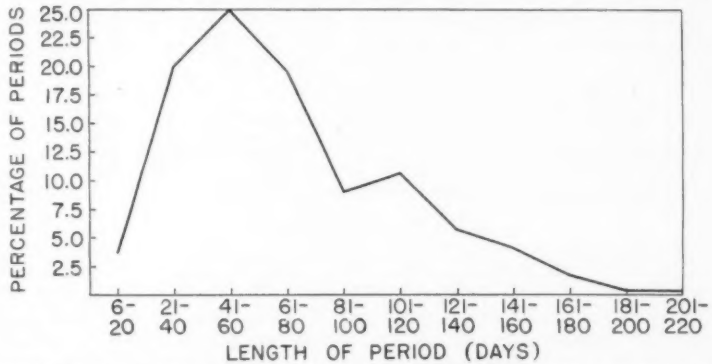

FIGURE 2.—Frequency polygon showing variation in length of period from parturition to first succeeding oestrus in clinically normal cattle

4 weeks is the usual time if the cow is milked, and about 3 months if the calf is suckled. The range given by stockmen was 9 days to 6 months.

The opinion of stockmen, insofar as the range is concerned, is closely paralleled by the records for the clinically normal cattle of the Wisconsin herd, considering only those post-partum intervals preceded by normal gestation periods (nonabortive). The distribution of the different lengths of the period from parturition to the first subsequent oestrus is given in table 9 and figure 2.

The skewness of this distribution needs no explanation further than that already given in connection with the distribution of oestrous cycles. That there is a need for close observation of the oestrous periods following parturition is apparent from the practical standpoint of having cows calve at least every 12 or 13 months. To accomplish this involves fertile service before 115 days following parturition.

CLINICAL ABNORMALITY

In the calving intervals preceding the ones in which cystic follicles or retained corpora lutea were noted, the period from parturition to first oestrus (preceded by normal gestation) was not significantly

different from the length of this period in the calving intervals following the ovarian disturbance, and neither of these figures differed significantly from that for the clinically normal animals (table 9). The variations within this period in calving intervals prior to the ensuing abnormality do not, therefore, foretell the subsequent disturbance, nor do the ones following the disturbance show the effects of it.

TABLE 9.—*Frequency distribution of the periods from parturition to first subsequent oestrus in normal and abnormal cattle*

Length of period (days)	Periods of clinically normal cows		Periods of clinically abnormal cows [1]		All periods	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1–20	8	4.5	1	2	9	3.7
21–40	33	18.4	16	25	49	20.1
41–60	50	27.9	11	17	61	25.0
61–80	32	17.9	15	23	47	19.3
81–100	15	8.4	7	11	22	9.0
101–120	20	11.2	6	9	26	10.7
121–140	11	6.1	3	5	14	5.7
141–160	6	3.4	4	6	10	4.1
161–180	2	1.1	2	3	4	1.6
181–200	1	.6	0	0	1	.4
201–220	1	.6	0	0	1	.4
Total	179		65		244	
Mean length of periods	*Days* 69.3±2.9		*Days* 71.4±4.9		*Days* 69.9±2.5	

[1] Preceding and following calving interval of abnormality.

INDIVIDUALITY

The gross variation of the periods from parturition to first subsequent oestrus, in calving intervals preceded by nonabortive gestations and of clinically normal cows, was divided into that between cows and that between calving intervals of the same cow. Within a fairly wide range of variation a cow tends to repeat a similar length of parturition to first subsequent oestrus in different calving intervals.

TABLE 10.—*Analysis of variance in period from parturition to first subsequent oestrus in clinically normal cattle*

Range (days)	Source of variation	Degrees of freedom	Sum of squares	Mean square	*P*	η^2
Entire	Individual	44	102,703	2,334.2	<0.01	0.19
	Remainder	118	146,574	1,242.2		
	Total	162	249,277	1,538.7		
<86	Individual	35	18,874	539.3	≐.04	.16
	Remainder	73	24,563	336.5		
	Total	108	43,437	402.2		
Entire	Season	5	3,829	765.8	>.05	
	Remainder	183	264,141	1,443.4		
	Total	188	267,970	1,425.4		
<86	Season	5	5,903	1,180.6	<.01	.08
	Remainder	129	46,592	361.2		
	Total	134	52,495	391.8		

About the same proportional amount of variation is associated with individuality in the entire range as in the restricted range of less than 86 days (table 10). This repeatability tendency means that the breeding efficiency of a cow is inherently lowered when this period in her reproductive cycle is so long that she cannot conceive in the required time following parturition.

SEASON

In the mare Satô and Hoshi (*35*) noted a tendency to shorter intervals from parturition to first subsequent oestrus as the season advanced from March to June.

The entire range of periods for clinically normal cows does not show any variation which can be attributed to seasonal changes, on the basis of a bimonthly classification, beginning with January. When, however, only those less than 86 days in length are considered, season is found to account for 8 percent of the variation (table 10). In the more typical cases, there is a definite tendency for the period from parturition to the first subsequent oestrus to be longer in the spring (March–April, May–June) than in other seasons of the year.

COMPONENT PHASES OF THE SEXUAL CYCLE

If the variation in length of the component phases of the sexual cycle can be attributed to determinable causes, additional evidence is given as to the type of association between ovarian, pituitary, etc., processes and the external and internal influences studied.

GENERAL VARIATION

The duration of oestrus was not recorded in the cattle upon which the first two sections of this report were largely based. It is therefore necessary to rely on published records for the raw material of this analysis of the component phases of the sexual cycle of the several species, except in the case of swine. Table 11 presents the statistics on the oestrous period, menstrual period, preovulatory (follicular) phase, and postovulatory (luteal) phase, based on the available data.

TABLE 11.—*Statistics of component phases of the sexual cycle*

Species	Reference	Phase	Number	Mean	Standard deviation
				Hours	*Hours*
Cattle	(*13*)	Oestrous period	54	16.2±0.7	5.2
Sheep	(*12*)	do	77	36.2±2.0	17.1
Do	(*34*)	do	59	43.9±1.8	14.5
Do	(*41*)	do	884	30.4±0.4	10.4
Swine	(1)	do	107	51. ±1.6	16.2
				Days	*Days*
Horse	(*35*)	do	1,179	7.5±.1	2.5
Monkey	(*6*)	Menstrual period	132	5.4±.2	1.8
Man	(*17*), (*11*)	do	1,024	4.7±.1	1.1
Monkey	(*16*)	Preovulatory	53	11.8±.3	2.0
Baboon	(*46*)	do	48	17.1±.5	3.3
Monkey	(*16*)	Postovulatory	53	13.5±.5	3.5
Baboon	(*46*)	do	48	15.1±.4	2.6

[1] McKENZIE, F. F. See footnote 5, p. 421.

There is inconsistency in the results of the experimental work on the causation of oestrus. There is, likewise, no complete agreement as to the time relation between oestrus and ovulation, though it would seem from the work of Aitken (*1*) and Seaborn (*36*) on the

mare, Lewis (*23*), and Corner and Amsbaugh (*7*) on the sow, Allen (*2*) on the mouse, Long and Evans (*24*) on the rat, Young et al. (*44*) on the guinea pig, Hammond (*13*), Pearl (*32*), Strodthoff (*39*), Murphey et al. (*29*) on the cow, McKenzie and Phillips (*26*), Quinlan and Maré (*34*) and Terrill (*41*) on the sheep, that, if ovulation occurs, it does so toward the end of or shortly after the termination of oestrus. It has, however, been demonstrated that oestrus sometimes occurs without ovulation and that ovulation may occur without observable oestrus.

In addition to the figures on oestrus given in table 11, Aitken (*1*) gives 4 to 11 days; and Hammond (*14*) 3 to 41 days, as the normal variation of oestrus in the mare, the average being approximately 7 days; Weber's figures (*43*) show a variation from 3 to 36 hours for cows; McKenzie (*25*) noted 1 to 3 days, and Struve (*40*) 2 to 4 days as the length of the oestrous period in sows. McKenzie and Phillips (*26*) give the average duration of oestrus in sheep as 26.8 hours, with a range of 5 to 50 hours.

The period of oestrus is poorly defined or is not expressed at all in the primates, whereas the uterine hemorrhage does, as a rule, sharply demarcate a sexual cycle. The general relationship between the ovarian processes and the menstrual flow has been outlined by many investigators, but there is not as yet complete agreement as to the specific causation of menstruation.

The recovery of tubal ova by Corner (*5*) in the monkey, and by Allen et al. (*4*) in man, the more recent work of rectal palpation of the monkey by Hartman (*16*), the physiological test of Knaus (*19*), and other observations, all point to the middle of the menstrual cycle as the usual time of ovulation. Hartman (*15*) and Corner (*6*), however, have records of frequent menstruation without ovulation in the monkey. But insofar as the menstrual periods are correlated with the ovarian cycle they are of just as much value as end points in a study of its periodicity as are the oestrous periods.

Analysis of the variation in the oestrous and menstrual cycles as a unit will not explain fully the mode of action of the associated variables. Where these variables localize, their activity can be learned more readily by analysis of the associated variation in restricted phases (follicular and luteal) of the cycle. The luteal phase is given as the time from ovulation to the beginning of menstruation; the follicular phase, from the beginning of menstruation to ovulation. These phases have been carefully recorded by Hartman (*16*) for the monkey, and by Zuckerman and Parkes (*46*) for the baboon. The general variation of the two phases of the sexual cycle for these species is shown in table 11.

Ogino (*30*) and Knaus (*19*) have stressed invariability of the luteal phase of the cycle in women and rabbits. The reports of Hartman, and Zuckerman and Parkes do not corroborate these conclusions for the monkey and baboon. They find both the luteal and follicular phases of the cycle quite variable. Hartman's records show a significantly longer (mean difference$=1.7\pm0.55$), as well as a significantly more variable (σ difference$=1.5\pm0.39$), postovulatory than preovulatory period. Zuckerman and Parkes' data on the baboon show a significantly longer (mean difference$=2.0\pm0.60$) follicular than luteal phase, and a slightly, but not significantly, greater variability (σ difference$=0.7\pm0.42$) for the follicular period.

INDIVIDUALITY

Individuality, as previously defined, is just as evident in the oestrous period of the four species studied as it is in the oestrous cycle (table 12). Likewise, the menstrual period in man and, to a lesser degree, in monkeys is found to be repeatable. On the basis of these data, individuality of the preovulatory phase of the cycle in monkeys and baboons cannot be established; there is, however, a slight indication of repeatability of this phase in the baboon. In the postovulatory period of the monkey, marked individuality is apparent. Forty-nine percent of the variation in this period is attributable to differences between individuals. In the baboon the differences between the intervariance and intravariances are again too small to be taken as more than the fluctuations of random sampling.

TABLE 12.—*Analysis of variance in component phases of sexual cycle*

Species	Reference	Phase	Grouping	Phases	Groups	P	η^2
				Number	*Number*		
Cattle [1]	(*13*)	Oestrous period	Individual	43	8	<0.01	0.44
Sheep	(*12*)	do	do	82	17	$<.01$	.34
Do	(*34*)	do	do	18	9	$>.05$	.45
Swine [2]	[3]	do	do	63	23	$<.01$	.50
Horse	(*35*)	do	do	50	11	$<.01$	.62
Man	(*17*)	Menstrual period	do	201	11	$<.01$	.63
Monkey	(*15*)	do	do	514	32	$<.01$	.17
Do	(*6*)	do	do	109	10	$<.05$	.07
Do	(*16*)	Preovulatory	do	37	13	$>.05$	------
Baboon	(*46*)	do	do	47	9	$<.05$	.20
Monkey	(*16*)	Postovulatory	do	37	13	$<.01$	.49
Baboon	(*46*)	do	do	47	9	$>.05$	.10
Cattle	(*13*)	Oestrous period	Age	54	2	$<.02$	.10
Swine	[3]	do	do	107	2	$<.01$	.58
Cattle [1]	(*13*)	do	Season	41	4	$>.05$	.09
Sheep	(*12*)	do	do	82	6	$>.05$	------

[1] Heifers.
[2] Gilts.
[3] McKENZIE, F. F. See footnote 5, p. 421.

AGE

McKenzie and Phillips (*26*) report a significantly longer oestrous period in yearling and in older ewes than in ewe lambs, and this has been confirmed by Terrill (*41*). Grant (*12*), on the other hand, found no correlation between age and duration of oestrus in sheep; neither did Satô and Hoshi (*35*) in horses. Hammond's records (*13*) point to a slightly longer period in cows than in heifers, and analysis of his records shows this to be significant. But there is strong probability of this being due to individuality. The sows had significantly longer and more variable periods than the gilts (table 12).

SEASON

Temporary suspension of oestrus occurs in various breeds of sheep during certain seasons of the year. Fluctuations in fertility in swine, cattle, horses, goats, etc., have been noted in correlation with seasonal changes.

Because of the small numbers with which we are dealing, it is not possible to establish whether there is a seasonal influence on duration of oestrus in either the heifers reported by Hammond or the sheep reported by Grant, although Hammond's figures give some indication

of a longer period for the summer than for the winter. In the records of Satô and Hoshi on the mare a consistent decrease was noted in the length of the oestrous period from March to July.

CORRELATIONS WITH SEXUAL CYCLE

Hammond found that experimental removal of the corpus luteum in the cow 6 or 7 days following oestrus not only shortened the cycle length, but also resulted in an abbreviated oestrous period. Marshall (*27*) found that in sheep the duration of oestrus and the length of the oestrous cycle are both curtailed when the sheep are brought from the highlands to the lowlands.

Hammond (*13*) noticed a general association between the cycle length and the duration of the immediately subsequent oestrus in his records but did not give an exact measure of it. Calculation of his data gives a highly significant positive correlation coefficient for oestrous cycle and subsequent oestrous period (table 13). The figures for sheep do not show any correlation of significance for a cycle either with its component oestrous period or with its immediately subsequent oestrous period, as pointed out by Grant (*12*). The swine data do not give evidence of any associations between the duration of these periods and cycle lengths.

TABLE 13.—*Correlation of sexual-cycle lengths with various component phases*

Species	Reference	Phase	Correlation calculated	Phases	Individuals	r	P
				Number	*Number*		
Cattle	(*13*)	Subsequent oestrus	Over all cycles of all individuals.	32	11	0.36	<0.01
Sheep	(*12*)	do	do	62	17	−.21	>.05
Swine	(1)	do	do	58	43	.06	>.05
Sheep	(*12*)	Component oestrus	do	65	17	.08	>.05
Swine	(1)	do	do	66	45	.03	>.05
Monkey	(*6*)	Component menstrual period.	Between individuals	109	10	.39	>.05
	(*6*)	do	Within individuals	109	10	.07	>.05
	(*6*)	do	Over all cycles of all individuals.	109	10	.09	>.05
	(*15*)	do	Between individuals	514	32	.49	<.01
	(*15*)	do	Within individuals	514	32	.08	>.05
	(*15*)	do	Over all cycles of all individuals.	514	32	.15	<.01
Baboon	(*16*)	Preovulatory	do	53	26	.28	÷.05
	(*16*)	Postovulatory	do	53	26	.84	<.01
	(*46*)	Preovulatory	do	48	10	.76	<.01
	(*46*)	Postovulatory	do	48	10	.56	<.01

[1] MCKENZIE, F. F. See footnote 5, p. 421.

Fluhmann (*11*), reporting menstrual cycles in women, divided his subjects on the basis of cycle variation into a "regular" and "irregular" group, and on the basis of cycle length into three subgroups within each of these. The analysis of the variation in duration of the menstrual period within and between the cycle-length classes gives a measure of the degree of correlation between the duration of the menstrual period and menstrual cycle length, at least so far as the average figures of a group of individuals are concerned. The association is found to be highly significant, but to account for only a small amount (7 percent) of the variation. The regular cycle group has a significantly longer menstrual period than the irregular group. The standard deviations of the menstrual periods of the two groups

differ significantly, thereby showing that variability in menstrual cycle and menstrual period are correlated. This correlation is positive.

Covariation between menstrual cycle and menstrual period could be more completely studied in Corner's and Hartman's records, because of the detailed protocols given in these papers. The size of the sample in one case (Corner) did not permit of decisive conclusions, but its similarity with the other sample (Hartman) of the monkey population can be seen in table 13.

The correlation coefficient ($r=0.15$) worked out for the 514 cycles and their component menstrual periods proves to be highly significant. Further analysis establishes the fact that this positive association is due to a correlation between the averages of these two variables per individual and not to any association between cycle length and its component period within each individual's lifetime. In other words, on an average, each individual has a typical cycle length which varies within small limits; similarly, each individual has a typical period duration which varies within small limits. These "cycle-length" and "period-duration" variations are not correlated within each individual's records; but the typical (or average) period of an individual and the average cycle tend to vary together. The degree of association is given by the regression coefficient (0.96) of cycle on its component period. In this case there is, on an average, approximately 1 day's increase in cycle length for every day's increase in period duration when applied to the means of the individuals' records.

The correlation between the length of the follicular (preovulatory) phase and the entire cycle for the monkey is small, but on the margin of statistical significance, while the correlation between the luteal phase and the cycle is large, very highly significant, and differs significantly from the other correlation. In the baboon the correlations are both significant, but not significantly different from each other. These correlations seem to be quite in accord with the other results found for the cycle and these two phases, that is, the high repeatability of both the cycle and postovulatory period, the lack of repetition of the preovulatory in the monkey, and the indications of repetition in the cycle and the luteal and follicular phases of the baboon.

SUMMARY

An attempt has been made to analyze the variations in the different phases of the reproductive cycle in such a way that their association with other recorded variables may be recognized.

Extreme variations in the lengths of the oestrous cycle of cattle not only indicate disturbances such as the presence of cystic follicles and retained corpora lutea but also serve as a warning of their occurrence in later calving intervals in those animals which are not known to be clinically abnormal at the time of these extreme cycle-length variations.

Potentially fertile matings are, on an average, followed by longer oestrous cycles in cattle, swine, and sheep than are services with a vasectomized male or no mating at all.

The differences between individuals in cattle, sheep, swine, and horses in the length of oestrous cycles, and in man, monkeys, and baboons in length of menstrual cycles, are the source of significantly

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more variation than the differences between the cycles of each individual except in immature monkeys, in which the variations of the cycle lengths of each individual are more marked than the variations in cycle lengths between individuals.

Slight increase in mean cycle length, but not in variation, is found with increasing age in cattle. No average cycle-length trends are apparent with increasing age in sheep, swine, horses, or monkeys. In man, however, there is a definite decrease in average cycle length and variation as menstrual life increases. The latter association, between variability of menstrual cycle and age, is also noted in monkeys.

No seasonal effects are evident in the cycle lengths of cattle or man. In one group of sheep, however, the length of the cycle increased as the breeding season advanced.

Clinical abnormality is not expressed in the variations of the period from parturition to first subsequent oestrus in calving intervals before and after the one in which abnormality has been recorded on the basis of manual rectal palpation of the ovaries. Individuals tend to repeat a similar length of parturition to first subsequent oestrus within fairly wide limits of variation. The length of this period does not show the effect of seasonal changes unless the range is restricted to include only those periods less than 86 days in length; on an average the longer periods were then found to occur in the spring.

Analysis of the published records on the length of the component phases shows that there is a very marked individuality in most of these in the different species studied. This was evident in the oestrous period of the cattle, sheep, swine, and horse, the menstrual period of man and monkey, and the postovulatory phase of the monkey. The preovulatory phase in the baboon showed no significant individual repeatability.

Average increase in the duration of oestrus in cattle and sheep with increasing age is strongly indicated; in swine a highly significant duration increase with age is noted.

No definite seasonal effect is evident in the duration of the oestrous periods of cattle and sheep.

There is a highly significant correlation between the length of the oestrous cycle and the duration of the subsequent oestrous period in cattle, but none was shown in sheep or swine.

A general correlation exists between duration of menstrual period and length of the cycle in man, and between these two (cycle and component period), for the means of individuals in the monkey, but none between these variables within individuals.

The postovulatory (luteal) phase is significantly more variable than the preovulatory (follicular) in the monkey, whereas both are equally variable in the baboon. The length of the luteal phase is highly correlated with cycle length in the monkey, and this correlation differs significantly from the low correlation between the length of the follicular phase and the cycle length. The lengths of both of these phases are correlated (to about the same degree) with cycle length in the baboon.

It is suggested that the factors found to be associated with the variation of the sexual cycle and its component phases be taken into account in analyses and interpretations made of these phenomena, with particular reference to (1) estimation of breeding efficiency and (2) planning and analyses of physiological experiments.

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RELATIVE EFFICIENCY FOR GROWING LAMBS OF THE PROTEIN IN RATIONS SUPPLEMENTED BY SOYBEAN-OIL MEAL, LINSEED MEAL, OR CORN-GLUTEN MEAL [1]

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INTRODUCTION

During the last 5 years this station has been conducting a series of nitrogen-balance experiments to determine the nutritive value of the protein in various rations for growing lambs. This work was undertaken because there is a decided lack of experimental data on this general problem in which ruminants have been used as experimental animals. Considering the economic importance of such livestock and the fact that ruminants may be entirely different from non-ruminants in their use of protein additional work seemed desirable.

The results of the preceding experiments have been reported in two publications from this station.[2] The first report showed no appreciable difference in the utilization of the proteins of alfalfa hay and clover hay when fed in nutritively complete rations to growing lambs. It showed also that there is no appreciable difference in efficiency between the proteins of an alfalfa and corn ration and the proteins of a clover and corn ration when fed at the same level of protein intake. As the biological values were practically the same for all four rations, the addition of corn proteins to clover and alfalfa proteins had no supplementary effect.

Additional work has recently been reported [3] from this station on the nutritive value of the proteins of soybean-oil meal, corn-gluten meal, and linseed meal when fed in such amounts as to furnish a protein level of 10 percent in addition to approximately 1 percent of protein in a low-nitrogen basal ration. When fed under such conditions to growing lambs, small but significantly higher biological values were obtained for the soybean-oil-meal ration. The average of the biological values was 72.8 for the protein in the soybean-oil-meal ration, 65.7 for the protein in the corn-gluten-meal ration, and 67.7 for the protein in the linseed-meal ration.

Since only slight differences were found in the nutritive value of the proteins in the rations in which one of the three protein supplements was fed as practically the sole source of protein, the question arose as to what differences, if any, would exist if these same supplements were fed in mixed rations containing proteins from several sources. Since apparently no nitrogen-balance experiments to solve this problem had been made the present series of experiments was undertaken.

[1] Received for publication Aug. 13, 1936; issued May, 1937. This paper is part of a thesis presented by John I. Miller to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. THE NUTRITIVE VALUE OF THE PROTEINS OF ALFALFA HAY AND CLOVER HAY WHEN FED ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN. Jour. Agr. Research 48: 555–570. 1934.

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[3] TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. See second reference in footnote 2.

No reports have been found in the literature of feeding experiments in which comparisons were made between these three meals when added to lamb rations which contained mostly nonleguminous roughages and which were therefore low in protein. Several feeding trials have been reported, however, in which comparisons were made of rations composed of corn and alfalfa hay supplemented with one of these three protein-rich feeds. In addition to the fact that the basal ration of corn and alfalfa hay alone is adequate in amount of protein and is an excellent ration for lambs, little or no attention was paid to the differences in the protein content of the supplements. Under these conditions as summarized by Morrison,[4] no differences in efficiency have been shown between soybean-oil meal, linseed meal, and corn-gluten meal for growing, fattening lambs.

In the experiments herein reported nitrogen-balance experiments were conducted with (1) lambs fed corn grain and timothy hay supplemented with each of these three protein-rich feeds and (2) with lambs fed similar rations except that corn stover replaced the timothy hay. This study with the corn-stover rations was of especial interest because the ration of corn grain, corn-gluten meal, and corn stover was entirely from the corn plant. Thus a comparison could be made between a ration containing protein from a single plant with others containing protein from different sources.

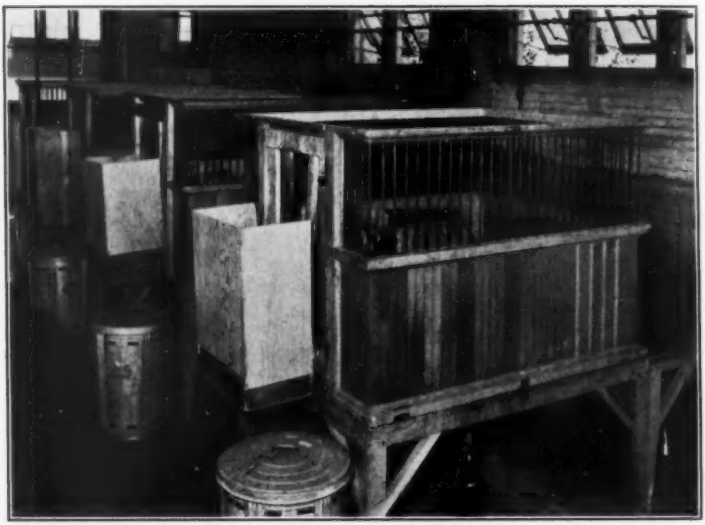

FIGURE 1.—Metabolism cages which permit separate collection of urine and feces.

EXPERIMENTAL PROCEDURE

The general procedure and methods of analysis followed in this work were practically the same as those followed in earlier work at this laboratory. Purebred or high-grade wether lambs of excellent mutton type were secured from the station flock and placed in metabolism cages (fig. 1). Urine and feces were collected daily during 10-day

[4] MORRISON, F. B. FEEDS AND FEEDING . . . Ed. 20, unabridged, 1050 pp., illus. Ithaca, N. Y. 1936.

collection periods, and from these collections the nitrogen balances were determined. All preliminary and intervening periods were 10 days or more in length.

No attempt was made to put the lambs on a low-nitrogen ration, as is customary in the determination of the biological values of proteins.[5] An effort was made to develop a low-nitrogen ration containing only purified ingredients, but the lambs would not consume a satisfactory amount of it for any appreciable length of time. Moreover, work at this laboratory [6] has indicated that periods of low-nitrogen feeding tend to promote increased nitrogen retention by lambs during the subsequent period of feeding. It was therefore thought that more reliable measurements of protein utilization might be made if the lambs were not forced to undergo this period of severe malnutrition.

The methods emphasized in this work for comparing the efficiency of the proteins are those of percentage retention of total nitrogen and of digested nitrogen. Existing data indicate that these methods are reliable and satisfactory. For purposes of comparison, however, biological values have been calculated for the proteins of the various rations. In making these calculations average values for endogenous and metabolic nitrogen as determined in 14 comparable experiments with lambs at this station [7] were used. These experiments represent an arbitrary selection of the data reported, as only those data were employed in which the dry-matter intake was at least 3,000 g for the 10-day periods. The average values for the 10-day periods were 0.37 g of endogenous nitrogen per kilogram of body weight and 0.552 g of metabolic nitrogen per 100 g of dry-matter intake.

SERIES 1: RATIONS WITH TIMOTHY HAY AS THE ROUGHAGE

The percentage composition of the feeds used in the first series of experiments is shown in table 1.

TABLE 1.—*Percentage composition of feeds used in experiments 1 and 2, series 1*

Experiment and feed	Water	Mineral matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Experiment 1:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Timothy hay	9.21	4.69	5.38	2.18	33.20	45.34
Corn, yellow	8.97	1.57	9.85	5.04	2.06	72.51
Corn-gluten meal	8.11	1.11	41.45	1.66	1.15	47.12
Soybean-oil meal	7.62	5.70	40.45	5.00	4.89	36.34
Linseed meal	8.62	4.94	33.19	4.43	7.64	41.18
Experiment 2:						
Timothy hay	10.66	4.76	6.93	2.47	32.26	42.92
Corn, yellow	11.99	1.30	9.13	3.87	1.90	71.81
Corn-gluten meal	6.37	1.31	41.81	3.43	3.88	43.20
Soybean-oil meal	6.11	6.16	41.14	5.90	3.58	37.11
Linseed meal	8.20	5.58	34.88	6.36	6.12	38.86

All the feeds were of good quality. The timothy hay fed throughout the first experiment was somewhat more mature than is desirable for lambs, but in the second experiment an excellent quality of hay cut in early bloom was fed. The concentrates were obtained from a local feed store and were representative of feeds commonly used in this section. The soybean-oil meal was produced by the expeller

[5] MITCHELL, H. H. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. Jour. Biol. Chem. 58: 873–903. 1924.

[6] TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. See second reference in footnote 2; also both references in footnote 2.

[7] See footnote 6.

process and had a definite cooked odor and a nutlike taste. In the second experiment, to make the rations more palatable, pea-sized linseed cake and soybean-oil cake were used in place of the respective meals. The percentage composition of the rations is given in table 2.

TABLE 2.—*Percentage composition and average protein content of rations fed in experiments 1 and 2, series 1*

Experiment and feed	Soybean-oil-meal ration	Linseed-meal ration	Corn-gluten-meal ration	Experiment and feed	Soybean-oil-meal ration	Linseed-meal ration	Corn-gluten-meal ration
Experiment 1:	*Percent*	*Percent*	*Percent*	Experiment 2:	*Percent*	*Percent*	*Percent*
Timothy hay	50.0	50.0	50.0	Timothy hay	50.0	50.0	50.0
Soybean-oil meal	8.4			Soybean-oil meal	6.5		
Linseed meal		11.0		Linseed meal		8.0	
Corn-gluten meal			8.3	Corn-gluten meal			6.4
Corn, yellow	40.6	38.0	40.3	Corn, yellow	42.5	41.0	42.2
Mineral	1.0	1.0	1.4	Mineral	1.0	1.0	1.4
Total	100.0	100.0	100.0	Total	100.0	100.0	100.0
Average protein content (N×6.25)	9.84	9.70	9.79	Average protein content (N×6.25)	10.47	10.62	10.34

The mineral mixture was composed of 40 percent of ground limestone, 40 percent of steamed bonemeal, and 20 percent of salt. It was fed in such amounts as to make up 1 percent of the total ration, except in the case of the corn-gluten-meal ration, in which it was 1.4 percent. This modification was made in order to keep the mineral content approximately the same for the three rations. The lambs were allowed free access to common salt during all preliminary and intervening periods.

The roughage made up 50 percent of the ration in each case and the corn and protein supplements were added in such proportions as to furnish approximately 10 percent of protein. The rations did not contain exactly the same percentages of fat and fiber, but the differences were so small that it was not believed that they would influence the results.

The lambs were fed twice daily throughout the experiments. The daily allowances of feed for the 10-day periods for each lamb were weighed into individual paper bags at the beginning of the periods and the moisture and nitrogen content determined. These bags were stored in tightly closed metal containers. In the first experiment, the hay was run through a hammer mill and mixed with the concentrates before feeding, but in the second experiment the hay was chopped in a feed cutter and fed separately in an effort to increase the palatability of the rations. The lambs were rotated on the various rations as is customary in such experiments.

Considerable difficulty was encountered in the first experiment in keeping the lambs on a satisfactory level of food intake, as the timothy hay did not prove palatable, due in part no doubt to the maturity of the hay when cut. In the second experiment no trouble was experienced in keeping the lambs on feed. However, to keep the feed consumption as uniform as possible, only such amounts were offered as it was believed would be readily consumed by all the lambs.

RESULTS

The data secured in the first two experiments are presented in table 3 and the final values are summarized in table 4.

TABLE 3.—*Nitrogen-balance data showing digestibility and utilization of proteins of the different rations in experiments 1 and 2,[1] series 1*

Ration and lamb no.	Body weight			Food intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Coefficient of apparent digestibility	Total nitrogen retained	Digestible nitrogen retained	Estimated biological value [2]
	Initial	Final	Average									
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
EXPERIMENT 1												
Soybean-oil meal:												
1	30.77	31.03	30.90	8,000	7,270.4	125.92	54.95	50.34	60.0	16.4	27.3	62
2	34.07	34.83	34.45	8,000	7,204.0	124.77	59.10	47.34	62.0	14.7	23.7	60
3 [3]	38.40	39.53	38.96	7,000	6,342.0	115.22	39.57	39.52	65.7	31.3	47.7	77
Linseed meal:												
1	30.20	30.70	30.45	6,525	5,852.9	109.71	37.06	41.93	61.8	28.0	45.3	74
2	31.50	32.20	31.85	8,000	7,240.0	128.20	49.28	46.14	64.0	25.6	40.0	70
3	32.40	33.00	32.70	8,000	7,236.8	124.16	59.60	50.67	59.2	11.2	18.9	58
Corn-gluten meal:												
1	32.13	32.80	32.46	6,625	6,069.2	104.94	40.07	37.48	64.3	26.1	40.6	72
2	32.40	33.00	32.70	8,000	7,249.6	125.35	53.23	48.21	61.5	19.1	30.9	65
3	34.50	34.77	34.64	7,500	6,739.5	116.53	63.11	38.21	67.2	13.1	19.4	56
EXPERIMENT 2												
Soybean-oil meal:												
4	27.67	28.57	28.12	6,000	5,374.0	100.13	38.16	28.68	71.4	33.2	46.6	72
5	25.17	25.57	25.37	6,000	5,380.8	102.59	51.28	30.96	69.8	19.8	28.4	59
6	32.33	32.90	32.62	7,000	6,297.2	114.52	74.53	24.52	78.6	13.5	17.2	45
7	28.10	28.53	28.32	7,000	6,297.2	114.52	63.63	33.21	71.0	15.4	21.7	54
8	29.50	30.17	29.84	7,000	6,302.8	120.02	53.58	35.87	70.1	25.5	36.3	64
Linseed meal:												
4	30.20	30.50	30.35	6,000	5,344.0	99.61	43.47	30.35	69.5	25.9	37.2	67
5	27.10	28.17	27.64	7,000	6,291.0	121.68	54.24	37.48	69.2	24.6	35.6	63
6	30.20	30.52	30.36	6,000	5,375.7	99.37	63.90	25.03	74.8	10.5	14.0	47
7	26.00	26.93	26.46	7,000	6,296.9	121.77	58.10	35.51	70.8	23.1	32.6	60
8	27.83	28.67	28.25	7,000	6,287.8	116.14	53.09	39.59	65.9	20.2	30.6	62
Corn-gluten meal:												
4	29.30	29.33	29.32	6,000	5,379.0	98.21	34.66	29.02	70.4	35.2	49.9	76
5	29.23	30.17	29.70	7,000	6,303.5	114.92	55.50	33.81	70.6	22.3	31.6	61
6	31.80	32.20	32.00	7,000	6,303.2	116.67	67.73	28.24	75.8	17.7	23.4	52
7	24.62	24.73	24.68	6,000	5,400.0	99.98	49.30	31.52	68.5	19.2	28.0	59
8	30.97	31.63	31.30	7,000	6,303.5	114.92	47.50	33.60	70.8	29.4	41.6	69

[1] Totals are for 10-day experimental periods.
[2] Calculated from average values for endogenous and metabolic nitrogen.
[3] Another lamb substituted for lamb no. 3 in this determination.

TABLE 4.—*Summary of digestibility and utilization of the proteins of the different rations in experiments 1 and 2, series 1*

Item	Experiment 1			Experiment 2					Average
	Lamb no. 1	Lamb no. 2	Lamb no. 3	Lamb no. 4	Lamb no. 5	Lamb no. 6	Lamb no. 7	Lamb no. 8	
Soybean-oil-meal ration:									
Coefficient of apparent digestibility.....percent..	60.0	62.0	65.7	71.4	69.8	78.6	71.0	70.1	68.6±1.40
Total nitrogen stored.do....	16.4	14.7	31.3	33.2	19.8	13.5	15.4	25.5	21.2±1.85
Digestible nitrogen stored percent..	27.3	23.7	47.7	46.6	28.4	17.2	21.7	36.3	31.1±2.70
Estimated biological value percent..	62	60	77	72	59	45	54	64	62 ±2.37
Linseed-meal ration:									
Coefficient of apparent digestibility.....percent..	61.8	64.0	59.2	69.5	69.2	74.8	70.8	65.9	66.9±1.22
Total nitrogen stored.do....	28.0	25.6	11.2	25.9	24.6	10.5	23.1	20.2	21.1±1.61
Digestible nitrogen stored percent..	45.3	40.0	18.9	37.2	35.6	14.0	32.6	30.6	31.8±2.51
Estimated biological value percent..	74	70	58	67	63	47	60	62	63 ±1.97
Corn-gluten-meal ration:									
Coefficient of apparent digestibility.....percent..	64.3	61.5	67.2	70.4	70.6	75.8	68.5	70.8	68.6±1.05
Total nitrogen stored.do....	26.1	19.1	13.1	35.2	22.3	17.7	19.2	29.4	22.8±1.70
Digestible nitrogen stored percent..	40.6	30.9	19.4	49.9	31.6	23.4	28.0	41.6	33.2±2.43
Estimated biological value percent..	72	65	56	76	61	52	59	69	64 ±1.97

Inspection of the data shows that in practically all respects there is reasonably close agreement in the average results obtained in the two experiments. It is apparent that considerable variations in individual values have occurred, for which no entirely satisfactory explanation can be given. However, variations in individual values are not uncommon in nitrogen-balance experiments with large animals.

There were no consistent differences in the apparent digestibility of the proteins of the three rations. The average digestibility was practically identical for each ration. These results are about what would be expected, since the rations were virtually the same, except for the protein furnished by the protein supplements. The average digestion coefficients of the proteins of these three meals as given by Morrison [8] are almost identical. However, the digestibility of the protein in the rations used in the first experiment was appreciably lower than that in the second experiment. This was no doubt due to the lower digestibility of the protein in the more mature timothy hay used in the first experiment.

The percentage of total nitrogen stored averaged 21.2 for the soybean-oil-meal ration, 21.1 for the linseed-meal ration, and 22.8 for the corn-gluten-meal ration. There were no consistent differences among the lambs in favor of any one ration, and obviously the small differences in the average percentage of total nitrogen stored cannot be considered significant. These data have been analyzed statistically by the analysis-of-variance method proposed by Fisher, Snedecor's factor F being used to determine the mathematical significance.[9] No significant differences were found between the means of the three different rations. In fact, there were considerably greater differences

[8] MORRISON, F. B. See footnote 4.
[9] LIVERMORE, J. R. LABORATORY EXERCISES IN STATISTICAL METHODS OF ANALYSIS. Cornell Univ., Dept. Plant Breeding. 1936.

between lambs than between any two rations. The average percentage of digestible nitrogen stored, as well as the estimated biological values for the three rations, also indicate that there were no appreciable differences in the efficiency of the proteins. Therefore, the three different expressions of protein utilization agree in this respect.

SERIES 2: RATIONS WITH CORN STOVER AS THE ROUGHAGE

The percentage composition of the feeds used in the second series of experiments is given in table 5.

TABLE 5.—*Percentage composition of feeds used in experiments 3 and 4, series 2*

Feed	Water	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Corn stover	10.93	4.45	5.95	1.62	28.48	48.57
Corn, yellow	11.99	1.30	9.13	3.87	1.90	71.81
Corn-gluten meal	6.37	1.31	41.81	3.43	3.88	43.20
Soybean-oil meal	6.11	6.16	41.14	5.90	3.58	37.11
Linseed meal	8.20	5.58	34.88	6.36	6.12	38.86

All the feeds except the corn stover were the same as those used in experiment 2. The stover used in most of the trials was fine-stalked and leafy, and therefore of excellent quality. The later trials (experiment 4) were conducted on the new season's corn stover. Because of unfavorable weather during the fall, this stover was of only fair quality. In each case the stover was run through a feed cutter and fed separately from the concentrates. The percentage composition of the rations is given in table 6.

TABLE 6.—*Percentage composition and average protein content of corn-stover rations used in experiments 3 and 4, series 2*

Feed	Soybean-oil-meal ration	Linseed-meal ration	Corn-gluten-meal ration
	Percent	*Percent*	*Percent*
Corn stover	50.0	50.0	50.0
Soybean-oil meal	8.0		
Linseed meal		10.0	
Corn-gluten meal			8.0
Corn, yellow	41.0	39.0	40.6
Mineral	1.0	1.0	1.4
Total	100.0	100.0	100.0
Average protein content (N×6.25)	10.48	10.56	10.36

RESULTS

The results of experiments 3 and 4 are presented in table 7 and are summarized in table 8.

The data are very similar to those obtained in the experiments with timothy hay. It will be noted, however, that there are a few unusually low values for nitrogen retention in the later trials. This was probably due to the change from the better cured, drier corn stover to the less palatable new season's stover, as the former level of feed consumption was not maintained during these periods. Since such values appear for each ration, and since the lambs were all under comparable conditions, it is believed that these values represent valid comparisons.

There was no difference in the average apparent digestibility of the protein of the three rations, a finding which is in complete agreement with that of the trials with timothy hay.

The average percentage of total nitrogen stored was 17.0 for the soybean-oil-meal ration, 15.9 for the linseed-meal ration, and 16.3 for the corn-gluten-meal ration. In addition to the fact that there were only slight differences in the average values for the three rations, no differences in protein efficiency were noted in the data for the individual lambs. When these data were analyzed statistically no significant differences between the means of any two rations were found.

TABLE 7.—*Nitrogen-balance data showing digestibility and utilization of proteins of the different rations in experiments 3 and 4,*[1] *series 2*

Ration and lamb no.	Body weight			Food intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Coefficient of apparent digestibility	Total nitrogen retained	Digestible nitrogen retained	Estimated biological value[2]
	Initial	Final	Average									
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
EXPERIMENT 3												
Soybean-oil meal:												
9	25.87	27.0	26.44	7,000	6,266.4	117.46	41.66	47.10	59.9	24.4	40.8	70
10	28.20	28.23	28.22	7,000	6,268.0	116.97	64.32	36.57	68.7	13.8	20.0	53
11	27.70	28.33	28.02	7,000	6,268.0	116.97	63.00	38.31	67.2	13.4	19.9	54
12	27.13	27.20	27.17	7,000	6,269.6	117.50	57.30	38.71	67.1	18.3	27.3	58
Linseed meal:												
9	27.87	28.40	28.14	7,000	6,257.6	119.80	46.62	46.26	61.4	22.5	36.6	66
10	25.90	26.47	26.18	7,000	6,272.6	116.21	60.51	38.27	67.1	15.0	22.4	55
11	26.67	27.43	27.05	7,000	6,275.8	120.01	62.41	36.61	69.5	17.5	25.2	56
12	25.23	26.32	25.78	7,000	6,338.1	116.91	55.30	41.90	64.2	16.9	26.3	58
Corn-gluten meal:												
9	29.80	30.27	30.04	7,000	6,265.3	112.71	40.30	43.74	61.2	25.4	41.6	72
10	27.27	27.50	27.38	7,000	6,249.2	118.79	66.48	37.01	68.8	12.8	18.7	52
11	25.62	26.28	25.95	7,000	6,329.7	119.66	54.70	40.23	66.4	20.7	31.1	61
12	27.27	27.90	27.58	7,000	6,265.3	112.71	53.89	39.85	64.6	16.8	26.0	59
EXPERIMENT 4												
Soybean-oil meal:												
5	30.17	30.30	30.24	7,000	6,267	117.01	62.10	36.48	68.8	15.8	22.9	56
6	35.07	35.03	35.05	6,000	5,166	93.60	52.29	22.88	75.6	19.7	26.1	58
7	29.17	29.53	29.35	6,000	5,179	94.66	45.37	31.96	66.2	18.3	27.6	62
8	32.77	33.17	32.97	6,000	4,949	100.03	58.90	29.20	70.8	11.9	16.8	52
Linseed meal:												
5	30.00	30.30	30.15	6,125	4,946	97.31	63.50	24.58	74.7	9.5	12.7	46
6	33.60	34.40	34.00	7,000	6,274	120.02	60.61	39.28	67.3	16.8	24.9	58
7	28.17	28.30	28.24	6,030	4,954	100.13	64.68	31.01	69.0	4.4	6.4	44
8	32.37	32.37	32.37	7,000	5,654	112.70	48.68	36.26	67.8	24.6	36.3	66
Corn-gluten meal:												
5	31.30	31.71	31.50	6,000	5,164	93.79	55.12	25.10	73.2	14.5	26.4	54
6	34.73	35.37	35.05	5,900	4,758	94.02	66.31	23.15	75.4	4.8	6.4	43
7	28.80	29.23	29.02	7,000	6,341	113.00	52.38	35.00	69.0	22.7	32.9	63
8	31.37	30.83	31.10	5,150	4,443	81.41	40.82	30.04	63.1	13.0	20.5	61

[1] Totals are for 10-day experimental periods.
[2] Calculated with average values for endogenous and metabolic nitrogen.

TABLE 8.—*Summary of digestibility and utilization of the proteins of the different rations in experiments 3 and 4, series 2*

Item	Experiment 3				Experiment 4				Average
	Lamb no. 9	Lamb no. 10	Lamb no. 11	Lamb no. 12	Lamb no. 5	Lamb no. 6	Lamb no. 7	Lamb no. 8	
Soybean-oil meal ration:									
Coefficient of apparent digestibility.....percent..	59.9	68.7	67.2	67.1	68.8	75.6	66.2	70.8	68.0±1.06
Total nitrogen stored.do....	24.4	13.8	13.4	18.3	15.8	19.7	18.3	11.9	17.0±0.97
Digestible nitrogen stored percent..	40.8	20.0	19.9	27.3	22.9	26.1	27.6	16.8	25.2±1.75
Estimated biological value percent..	70	53	54	58	56	58	62	52	58.0±1.40
Linseed-meal ration:									
Coefficient of apparent digestibility.....percent..	61.4	67.1	69.5	64.2	74.7	67.3	69.0	67.8	67.6±0.94
Total nitrogen stored.do....	22.5	15	17.5	16.9	9.5	16.8	4.4	24.6	15.9±1.56
Digestible nitrgen stored percent..	36.6	22.4	25.2	26.3	12.7	24.9	6.4	36.3	23.8±2.66
Estimated biological value percent..	66	55	56	58	46	58	44	66	56 ±1.92
Corn-gluten-meal ration:									
Coefficient of apparent digestibility.....percent..	61.2	68.8	66.4	64.6	73.2	75.4	69.0	63.1	67.7±1.17
Total nitrogen stored.do....	25.4	12.8	20.7	16.8	14.5	4.8	22.7	13.0	16.3±1.56
Digestible nitrogen stored percent..	41.6	18.7	31.1	26.0	26.4	6.4	32.9	20.5	25.4±2.52
Estimated biological value percent..	72	52	61	59	54	43	63	61	58 ±2.12

The average percentages of digestible nitrogen stored and also the average estimated biological values were practically the same for each ration. This would be expected from the fact that no differences had been found in the digestibility or in the percentage of total nitrogen stored.

It is evident that the corn-gluten-meal ration containing protein entirely from the corn plant was just as efficient as the two rations which contained protein from other sources. This is of especial interest since it has been sometimes assumed that the protein in a ration made up entirely from one plant source (including both seed and forage) is likely to be lower in value than that in a ration made up from more than one species of plant. However, the question has apparently not been studied as to whether the proteins in the leaves and stems of a certain plant may have the same deficiencies as in the proteins in the seeds of the same plant.

It may be noted that there was in general a lower utilization of the proteins of the three corn-stover rations than of the three timothy-hay rations. This might possibly indicate that corn stover is a less efficient source of protein in these combinations of feeds than is timothy hay. The data, however, furnish only indirect evidence since the experiments were not planned to compare directly the efficiency of the proteins of the two roughages when fed in mixed rations.

DISCUSSION

The data presented in this paper comparing the relative nitrogen utilization in two low-protein basal rations supplemented with soybean-oil meal, linseed meal, or corn-gluten meal have shown no appreciable differences when measured by nitrogen balances with growing lambs. In the earlier work at this station already referred to, a ration in which practically all the protein was furnished by soybean-oil meal was

found to be slightly, but measurably, superior to the protein of similar rations containing linseed meal or corn-gluten meal. This difference was not apparent in the experiments in which the protein-rich supplements furnished only enough protein to balance a ration of corn and timothy hay or corn and corn stover; that is, a ration in which the protein furnished by the supplements was about one-fourth to one-third of the total protein in the rations. It therefore seems probable that this slight difference in nutritive value was not able to express itself when decidedly less of the total protein in the ration was furnished by each of the three supplements, or that the mixture of proteins represented in each complete ration had no appreciable differences in efficiency.

While the nitrogen-balance experiments with lambs reported in this paper were carefully planned with the idea of detecting possible differences in the efficiency of the protein in three protein-rich concentrates as supplements to low protein rations, no differences were found. In the writer's opinion, such results should be interpreted as indicating no measurable differences in the protein utilization of the three rations with either corn stover or timothy hay, when fed under the conditions of these experiments. No statement as to what the results would be under practical conditions of lamb feeding on these rations can be made until properly planned experiments have been conducted to specifically study this problem. Obviously animals are under radically different conditions in a nitrogen-balance experiment than when fed under practical conditions of feeding.

On the basis of the results obtained in the experiments reported, one would not expect any appreciable differences in these rations due to differences in efficiency of protein utilization so long as they were fed under comparable conditions of protein intake. However, it is entirely possible that certain other factors might enter in that would cause appreciable differences in returns from lamb feeding, since such returns are in reality a measure of the "over-all" effect of a ration. None of the rations used in these experiments are considered to be very satisfactory lamb-fattening rations. This may possibly be due in part to the fact that rations containing the usual quality of timothy hay or corn stover are not so palatable for lambs as rations containing legume hay or silage as the roughage.

It was observed that the corn-gluten-meal rations were somewhat less palatable than the other rations. This difference in palatability may be important under the usual conditions of feeding in which high feed consumption is necessary for the proper finish at the desired market weights. Differences in palatability are not considered when an effort is made to control the feed intake in order that the animals may consume equal amounts of each ration and in such amounts that even the least palatable ration is consumed with no waste. Such conditions are desirable in nitrogen-balance experiments but are not obtained or even desired under practical conditions of feeding. It is believed that any appreciable differences in palatability is an important consideration, because in many cases such differences may be an expression of differences in the nutritive value of the rations.

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SUMMARY

Nitrogen-balance experiments have been conducted with eight growing lambs in a study of the digestibility and utilization of the protein in rations in which soybean-oil meal, linseed meal, or corn-gluten meal was used as a supplement to corn and timothy hay. The timothy hay made up 50 percent of the total ration and the corn and protein supplement were added in such proportions that there was approximately 10 percent of total protein in the ration. Eight nitrogen balances of 10 days' duration were conducted on each ration.

In these experiments there were no appreciable differences in the average apparent digestibility of the three rations; in the percentage of total nitrogen stored; in the percentage of digested nitrogen stored; or in the estimated biological value of the protein. The average percentage of total nitrogen stored was 21.2 for the soybean-oil-meal ration, 21.1 for the linseed-meal ration, and 22.8 for the corn-gluten-meal ration. There was no evidence that any one ration was superior to the others in efficiency of proteins.

In another series of nitrogen-balance experiments similarly conducted, a study was made on the same feeds except that corn stover replaced the timothy hay in the ration. Eight determinations were also made on each ration.

The average apparent digestibility was the same for each ration in which soybean-oil meal, linseed meal, or corn-gluten meal was used as a supplement to corn and corn stover. This was in complete agreement with the experiments on the timothy-hay rations. Moreover, there were no significant differences in the percentage of total nitrogen stored, in the percentage of digested nitrogen stored, or in the estimated biological value. The average percentage of total nitrogen stored was 17.0 for the soybean-oil-meal ration, 15.9 for the linseed-meal ration, and 16.3 for the corn-gluten-meal ration. Apparently, each ration permitted the same efficiency of protein utilization even though the three different protein supplements were used.

The results obtained in these two series of experiments with growing lambs indicate that soybean-oil meal, linseed meal, and corn-gluten meal have the same efficiency as supplements to a low-protein basal ration of corn and timothy hay or a ration of corn and corn stover insofar as the protein utilization of the total ration is concerned.

ANALYSIS OF COVARIANCE OF YIELD AND TIME TO FIRST SILKS IN MAIZE[1]

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INTRODUCTION

In the course of his investigations of heredity in numerous selfed lines from 14 varieties of maize (*Zea mays* L.), Jenkins (*5*)[3] examined the relation between yield and the time elapsing after June 30 to the first appearance of silks. The 14 varieties, developed at various latitudes in Iowa, were all planted at Ames, near the center of the State. Each inbred line was grown in three replications, the corresponding datum recorded in table 1 below being their mean. The present probem is to determine the relation between the two variates, yield and time to silking, in the inbred lines as well as in the varieties themselves.

The analysis of the covariance of yield and time to first silks was reported earlier by Snedecor (*6*). However, recently so much progress has been made in the technique of covariance, that a reexamination of the data seems desirable. This is especially appropriate because covariance is proving to be an extremely valuable statistical method in biological research. Since the introduction of the technique by Fisher, it has been expanded by him (*3*, *4*) and his co-workers (*1*, *9*). A number of studies have been reported both in field trials and in experiments with animals. This article is designed to place before its readers some of the recent advances in covariance methodology.

DATA

The data on yield and days to first silks are displayed in table 1. As is usual in such computations there are required the sums, sums of squares, and sums of products of the observed values in each variety. These are recorded in the last three columns of the table. As examples, consider the data for the variety Silver King. The sum for time to silking is

$$24+31+26+30=111.$$

The sum of squares for yields is

$$(7.7)^2+(5.4)^2+(5.2)^2+(4.0)^2=131.49.$$

The sum of products is

$$(24)(7.7)+(31)(5.4)+(26)(5.2)+(30)(4.0)=607.4.$$

[1] Received for publication Oct. 16, 1936; issued May, 1937. Journal Paper no. J-381 of the Iowa Agricultural Experiment Station. Project no. 514.

[2] The authors express appreciation to M. T. Jenkins and A. A. Bryan, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for guidance in the biological interpretations recorded in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 459.

TABLE 1.—*Mean time to first silks together with mean yield of 3 15-hill plots of corn planted to 14 varities*

Variety	Number of inbred lines	Silks or yield	Period after June 30 to first silks, and shelled corn per plot												Sum	Mean	Sum of squares	Sum of products
Four County White	24	Silks....days	28	26	29	31	26	29	26	28	25	27	25	28				
		Yield....pounds	7.3	5.7	5.2	6.8	4.5	7.8	4.2	7.4	7.4	7.9	7.4	5.9				
Silver King	4	Silks....days	35	26	30	29	31	31	30	30	29	28	26	28	681	28.38	19,451	
		Yield....pounds	5.0	5.9	6.8	6.4	4.8	6.3	5.0	6.1	7.9	5.7	6.4	6.4	150.2	6.26	968.06	4,251.7
U. S. Selection 133	7	Silks....days	24	31	26	30									111	27.75	3,113	
		Yield....pounds	7.7	5.4	5.2	4.0									22.3	5.58	131.49	607.4
		Silks....days	30	25	29	39	27	32	23						205	29.29	6,169	
		Yield....pounds	2.8	4.7	3.7	2.6	8.4	1.8	5.0						29.0	4.14	149.18	809.6
Iodent	34	Silks....days	38	36	36	35	35	37	35	31	33	34	37	33				
		Yield....pounds	6.9	7.4	5.6	6.8	9.5	5.5	8.6	7.6	9.5	7.6	8.1	9.9				
		Silks....days	33	33	29	32	30	35	30	34	36	30	33					
		Yield....pounds	5.8	9.4	6.7	8.4	12.0	9.4	15.9	7.4	6.6	5.4	8.4					
U. S. Selection 204	5	Silks....days	30	30	34	32	35	36	38	31	32	30	31		1,134	33.35	38,044	
		Yield....pounds	7.3	7.1	8.9	10.0	9.0	5.2	10.8	3.9	9.2	8.6	12.2		280.6	8.25	2,494.72	9,316.9
Lancaster Surecrop	11	Silks....days	36	36	38	38	39								187	37.40	7,001	
		Yield....pounds	7.0	6.5	2.3	4.5	5.7								26.0	5.20	149.28	966.7
Black Yellow Dent	8	Silks....days	33	33	32	36	33	38	30	38	31	32	32		368	33.45	12 384	
		Yield....pounds	9.6	7.8	9.6	7.7	8.2	7.3	11.3	9.5	8.8	8.4	6.8		95.0	8.64	837.16	3,165.8
Proudfit Yellow Dent	6	Silks....days	34	38	33	32	36	35	34	34					276	34.50	9,546	
		Yield....pounds	11.6	7.4	11.6	9.6	9.3	4.8	4.2	4.3					62.8	7.85	561.70	2,157.4
Krizer Bros. Yellow Dent	7	Silks....days	34	36	34	36	36	40							216	36.00	7,800	
		Yield....pounds	6.8	10.4	8.4	9.6	9.0	8.6							52.8	8.80	472.08	1,904.8
McCulloch Yellow Dent	7	Silks....days	35	30	43	35	35	32	30						240	34.29	8,348	
		Yield....pounds	7.6	7.9	6.8	8.6	6.0	5.3	8.4						50.6	7.23	375.02	1,728.0
Osterland Yellow Dent	7	Silks....days	36	29	39	39	38	38	34						253	36.14	9,223	
		Yield....pounds	7.7	6.5	8.5	6.4	6.4	9.0	5.9						50.4	7.20	371.52	1,832.6
Clark Yellow Dent	7	Silks....days	31	33	33	33	27	32	36						225	32.14	7,277	
		Yield....pounds	4.8	9.2	8.5	8.8	9.2	7.9	5.9						54.3	7.76	439.23	1,736.9
Walden Dent	7	Silks....days	27	33	25	35	27	27	26						200	28.57	5,802	
		Yield....pounds	4.3	8.4	6.6	4.9	5.8	7.6	3.7						41.3	5.90	261.71	1,187.8
Argentine Flint	8	Silks....days	36	37	34	41	33	33	29						243	34.71	8 521	
		Yield....pounds	5.8	6.5	10.8	2.0	5.6	8.5	9.6						48.8	6.97	392.30	1,642.2
Total (on 14 varieties)	142	Silks....days	42	40	43	34	29	30	33	27					278	34.75	9,928	
		Yield....pounds	2.3	4.9	2.9	5.3	5.3	5.3	2.8	2.6					31.4	3.92	136.58	1,072.8
		Silks....days													4,617	32.51	152,607	
		Yield....pounds													995.5	7.01	7,740.03	32,380.6

In many cases it is advantageous to "code" the data in order to simplify the computation. The number 20 or 30 might well have been subtracted from each number of days to first silks. With a calculating machine available, however, there would have been no net gain of time in the present computations.

Since it is sums of squares and products of deviations from mean that are required, it is necessary to "correct" the varietal sums of squares and sum of products of the two variates. For each variety three correction terms are necessary, those for Silver King being—

For time to silks, $(111)^2/4 = 3,080.25$,
For yield, $(22.3)^2/4 = 124.32$,
For products, $(111)\ (22.3)/4 = 618.82$.

The divisor 4 is the number of inbred lines in the variety; that is, the number of items added to form the sum. The sums of squares and products of deviations from means in this variety are calculated in this way—

For time to silks, $3,113.00 - 3,080.25 = 32.75$,
For yield, $131.49 - 124.32 = 7.17$,
For products, $607.40 - 618.82 = -11.42$.

These results for Silver King, together with the corresponding sums for the other varieties, are entered in table 2. From these statistics may be computed most of the desired information about the relation between yield and time to first silks.

TABLE 2.—*Sums of squares and products, correlation and regression coefficients, reduction in sum of squares, and sum of squares of errors of estimate in time to first silks and yield of 14 varieties of maize*

Variety	Degrees of freedom	Sum of squares and products			Correlation coefficient $\frac{Sxy}{\sqrt{(Sx^2)\ (Sy^2)}}$	Regression of yield on time to silks Sxy/Sx^2	Reduction in sum of squares $(Sxy)^2/Sx^2$	Errors of estimate	
		Time to silks Sx^2	Products Sxy	Yield Sy^2				Sum of squares $Sy^2-[(Sxy)^2/Sx^2]$	Degrees of freedom
Four County White	23	127.62	−10.22	28.06	−0.1708	−0.0801	0.82	27.24	22
Silver King	3	32.75	−11.42	7.17	−.7454	−.3487	3.98	3.19	2
U. S. Selection 133	6	165.43	−39.69	29.04	−.5726	−.2399	9.52	19.52	5
Iodent	33	221.76	−41.94	178.94	−.2105	−.1891	7.93	171.01	32
U. S. Selection 204	4	7.20	−5.70	14.08	−.5660	−.7917	4.51	9.57	3
Lancaster Surecrop	10	72.73	−12.38	16.71	−.3551	−.1702	2.11	14.60	9
Black Yellow Dent	7	24.00	−9.20	68.72	−.2265	−.3833	3.53	65.19	6
Proudfit Yellow Dent	5	24.00	4.00	7.44	.2994	.1667	.67	6.77	4
Krizer Bros. Yellow Dent	6	119.43	−6.86	9.25	−.2064	−.0574	.39	8.86	5
McCulloch Yellow Dent	6	78.86	11.00	8.64	.4215	.1395	1.53	7.11	5
Osterland Yellow Dent	6	44.86	−8.46	18.02	−.2976	−.1886	1.60	16.42	5
Clark Yellow Dent	6	87.71	7.80	18.04	.1961	.0889	.69	17.35	5
Walden Dent	6	85.43	−51.86	52.09	−.7774	−.6070	31.48	20.61	5
Argentine Flint	7	267.50	−18.35	13.34	−.3072	−.0686	1.26	12.08	6
Sum	128	1,359.28	−193.28	469.54				399.52	114

First, there are calculated in the usual manner the correlation and regression coefficients (*8*) recorded in the table. Turning again to

Silver King as an example, the correlation between yield and time to silking in this variety is

$$\frac{-11.42}{\sqrt{(32.75)\ (7.17)}}=-0.7454.$$

The regression of yield on time to first silks is

$$(-11.42)/(32.75)=-0.3487.$$

The regression coefficients in the several varieties vary from 0.1667 to −0.7917, 11 of them being negative. With the small number of degrees of freedom in most of the varieties, a good deal of sampling variation may be expected. One of the problems to be solved below is whether this observed variation among the regression coefficients

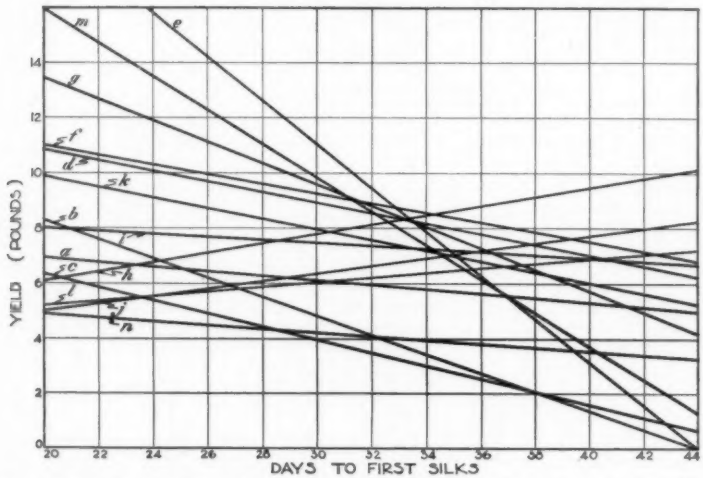

FIGURE 1.—Regressions of yield on days to first silks in 14 varieties of maize: *a*, Four County White; *b*, Silver King; *c*, U. S. Selection 133; *d*, Iodent; *e*, U. S. Selection 204; *f*, Lancaster Surecrop; *g*, Black Yellow Dent; *h*, Proudfit Yellow Dent; *i*, Krizer Bros. Yellow Dent; *j*, McCulloch Yellow Dent; *k*, Osterland Yellow Dent; *l*, Clark Yellow Dent; *m*, Walden Dent; *n*, Argentine Flint.

is significant. Since significant regressions imply significant correlations when there are only two variables, the same statement as above may be made about correlations.

The regression equations for the 14 varieties are plotted in figure 1. The equation for Silver King is

$$\begin{aligned}\overline{Y}&=5.58-0.3487(X-27.75),\\ &=-0.3487\ X+15.26,\end{aligned}$$

in which 5.58 pounds per plot is the mean yield for Silver King, and 27.75 days is the mean time to silking. In most of the varieties the tendency is for yield to increase with decreased time to the appearance of first silks. This seems reasonable, since both yield and earliness in these inbred lines may be considered evidences of vigor.

Certainly part of the variation in the yields of the inbred lines is associated with the regression of yield on time to silking. How great a part? The next step is directed toward the isolation of this portion from the total variance of the yields. The method is familiar to users of correlation analysis (*8, p. 17*), where r^2Sy^2 and $(1-r^2)Sy^2$ are the two parts of the sum of squares attributable respectively to regression and other causes. In the actual calculation, the value of r^2 is unnecessary. Since

$$r^2Sy^2=[(Sxy)^2/(Sx^2)(Sy^2)]\ (Sy^2)=(Sxy)^2/Sx^2,$$

it is sufficient to have the latter two quantities, $(Sxy)^2$ and Sx^2. Thus for Silver King, the reduction in sum of squares due to regression is

$$(Sxy)^2/Sx^2=(-11.42)^2/(32.75)=3.98,$$

the result being entered in the table. This is the part of the variation in strain yields associated with or caused by variation in earliness.

The remainder, $Sy^2-[(Sxy)^2/Sx^2]$, is the portion of the variation in yield not explained by regression; it is the sum of the squares of the errors of estimate. In the case of Silver King, this portion,

$$7.17-3.98=3.19,$$

is almost equal to the reduction ascribable to regression. This part of the variation in yield is independent of time to first silks. Presumably, this amount would be present even if all the strains silked at the same date. In the remainder of the table, comparison of the two portions of the sums of squares of variety yields, reduction, and remainder, gives an idea of the differing effects of regression in these groups of inbred lines. In Walden Dent more than half of the sum of the squares is associated with earliness, but in most of the varieties the fraction is decidedly smaller.

The degrees of freedom in the second column of table 2 are those applying to the sums of squares of yield and time to first silks. In each case, the degrees of freedom are less by one than the number of inbred lines. The deducted degree of freedom is associated with the mean which has been used in the computation. In the last column are the degrees of freedom for the sum of squares in the next preceding column. The number, in each case, is less by 2 than the number of inbred lines in the variety. The second degree of freedom to be deducted corresponds to the regression (or correlation) coefficient used in computing the errors of estimate.

One can now see clearly that the reduction in sum of squares owing to regression cannot be directly interpreted. The corresponding change in degrees of freedom must be taken into account. In a sample like Silver King the remaining sum of squares, less than half the original, is accompanied by only two degrees of freedom. The mean square error of estimate is therefore $3.19/2=1.60$. In testing the significance of the regression, this figure is directly comparable with the reduction in sum of squares, 3.98, with only one degree of freedom. The ratio, $F=3.98/1.60=2.5$, is not significant. From a different viewpoint, that of assessing the effect of regression, the mean square, 1.60, may be compared with the original mean square for

yield, 7.17/3=2.39, indicating a reduction in variance of only (2.39−1.60)/2.39=33 percent.

The sums of the last two columns of table 2 contributed valuable information. These are the pooled sums of squares of errors of estimate and degrees of freedom for all varieties, each error of estimate being measured from the individual variety regression. These sums are entered in the second line of table 3 and the mean square calculated,

$$399.52/114=3.50.$$

TABLE 3.—*Analysis of errors of estimate within varieties*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Within varieties, from average regression	127	442.05	3.48
Within varieties, from individual regressions	114	399.52	3.50
Remainder, between variety regression coefficients	13	42.53	3.27

This mean square is an average of that variation in yields not explained either by variety differences or by regression. It is therefore attributed to experimental error, and is the appropriate datum with which the magnitude of other mean squares may be compared.

The sums of the columns headed Sx^2, Sxy, and Sy^2 in table 2 are the pooled sums of squares and products for all varieties, each deviation being measured from the variety mean. These sums specify an average "within-variety" regression,

$$Sxy/Sx^2=(-193.28)/(1,359.28)=-0.1422,$$

and an average correlation,

$$\frac{Sxy}{\sqrt{(Sx^2)(Sy^2)}}=\frac{-193.28}{\sqrt{(1,359.28)(469.54)}}=-0.2419.$$

The corresponding regression equation is,

$$\begin{aligned}\overline{Y}&=7.01-0.1422\ (X-32.51),\\&=-0.1422\ X+11.63,\end{aligned}$$

where the means from the entire experiment are taken from table 1. The graph of this equation is plotted in figure 2. The corresponding within-variety correlation, −0.2419, is the appropriate average of the 14 variety correlation coefficients of table 2.

The yields of the inbred lines deviate from the within-variety regression more, in the aggregate, than from their individual variety regressions. The magnitude of the difference between the two sets of errors of estimate is easily computed. The sum of squares of the errors of estimate from the average regression is

$$469.54-[(193.28)^2/(1,359.28)]=442.05.$$

This within-varieties sum of squares of errors of estimate which is adjusted for the average within-variety regression is entered in table 3. The required difference is, then,

$$442.05-399.52=42.53.$$

This remainder in sum of squares is due to the failure of the individual variety regressions to coincide with the average within-variety regression. It is entered in table 3 along with the associated degrees of freedom. The resulting mean square (3.27) differs only slightly from the error mean square (3.50). The conclusion to be drawn is that the 14 variety regressions are not significantly different; that is, they differ from their average about as one would expect if they were drawn at random from a homogeneous population. In such a postulated population the variety regressions are all equal. The best available estimate of this common population regression is that within varieties. The degrees of freedom for this average regression are less by one than the sum in the second column of table 2. The one deducted corresponds to the regression coefficient, -0.1422, used in calculating the errors of estimate.

In the preceding paragraph a statement was made about the deviations of the yields of the inbred lines from the average regression. To be directly applicable the statement needs a slight qualification which can best be explained by references to the graphs in figures 1 and 2. In the latter figure is plotted the average within-variety regression equation. This graph passes through the point, mean days to silking=32.51 and mean yield=7.01, and has the slope -0.1422. Now, imagine the regression lines of figure 1 all passing through this same point. Each would retain its slope, but would be moved up or down till it contained the point (32.51, 7.01). Any differences among the varietal means would thus be eliminated from consideration. This is exactly what has been done in the foregoing calculations. The mechanism used was this: In each variety the sums of squares and products were those of deviations from the varietal means. The effect is the same as if each observed time and yield had been "corrected" by addition or subtraction so as to make the varietal means equal to the common experimental means, 32.51 and 7.01. It was noted above that even when this has been done, the pooled sum of squares of deviations from the variety regressions is less than the sum of the squares of all deviations from the average within-variety regression.

The next problem is to determine the behavior of the means of the 14 varieties. Mean earliness may be correlated with mean yield. The regression of mean yield on mean time to silks may or may not be significant. This regression may be the same as the average within-variety regression or it may be significantly different. These points and others are now to be considered.

The sums of squares and products appropriate for means may be calculated in the familiar manner (*6, example 2*), using the sums in table 1. For example, the sum of products is

$$\frac{(681)(150.2)}{24}+\frac{(111)(22.3)}{4}+\cdots$$

$$+\frac{(278)(31.4)}{8}-\frac{(4617)(995.5)}{142}=206.11.$$

But a simpler method is made available by the computations already completed. From the totals in table 1 it is easy to derive the two sums of squares and the sum of products for the whole experiment. These are—

$$\begin{aligned}
&\text{For time to silks,} && 152{,}607-[(4{,}617)^2/142] && =2{,}489.47.\\
&\text{For yield,} && 7{,}740.03-[(995.5)^2/142] && = 761.01.\\
&\text{For products,} && 32{,}380.6-[(4{,}617)(995.5)/142] && = 12.83.
\end{aligned}$$

The results are entered in table 4. Below them are the "within-variety" totals copied from the last line of table 2. The differences are the sums of squares and products between means of varieties, the sum of products being the same as that got by the longer method shown above. The indicated coefficients and sums of squares of errors of estimate are computed as before. The appropriate results are transferred to the summary table 5. Also, for reference, the sums of squares relating to yield are entered in the same table.

TABLE 4.—*Sums of squares and products, correlation and regression coefficients, and sum of squares of errors of estimate in time to first silks and yield in maize*

Source of variation	Degrees of freedom	Sums of squares and products			Correlation coefficient	Regression of yield on time to silks	Errors of estimate	
		Time to silks Sx^2	Products Sxy	Yield Sy^2			Sum of squares $Sy^2-[(Sxy)^2/Sx^2]$	Degrees of freedom
Total	141	2,489.47	12.83	761.01	0.0093	0.0052	760.94	140
Within varieties	128	1,359.28	−193.28	469.54	−.2419	−.1422	442.05	127
Between means of varieties	13	1,130.19	206.11	291.47	.3591	.1824	253.88	12

TABLE 5.—*Analysis of variance of yield and of errors of estimate in 14 varieties of maize*

Source of variation	Yield			Errors of estimate		
	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square
Within variety, from individual regressions				114	399.52	3.50
Between variety regressions				13	42.53	3.27
Within variety (table 4)	128	469.54	3.67			
Between means of varieties (table 4)	13	291.47	[1] 22.42	12	253.88	[1] 21.16
Within vs. between regressions				1	65.00	[1] 65.00
Total	141	761.01		140	760.94	

[1] Highly significant.

The equation for the regression of mean yield on mean days to first silks is

$$\begin{aligned}
\overline{Y} &= 7.01+.1824\ (X-32.51),\\
&= 0.1824\ X+1.08.
\end{aligned}$$

This equation is plotted in figure 2. The upward trend of the graph of this equation of means contrasts with the downward sloping graph

of the within-variety equation. While, with only 12 degrees of freedom, the regression of means is not significant (*3, p. 129; or 6, p. 67*), it nevertheless has a reasonable interpretation. The tested varieties were selected from both northern and central Iowa, but grown in the latter section only. Those from the north tended to early maturity, thus failing to enjoy the advantages of the longer growing season available. The varieties more closely adapted to the climate, while starting slowly, still had ample time to produce a good crop before frost.

This reasoning, however, is not so strongly supported by the experimental evidence as it would be if the regression coefficient were significant. The variety means are plotted in figure 2. The large

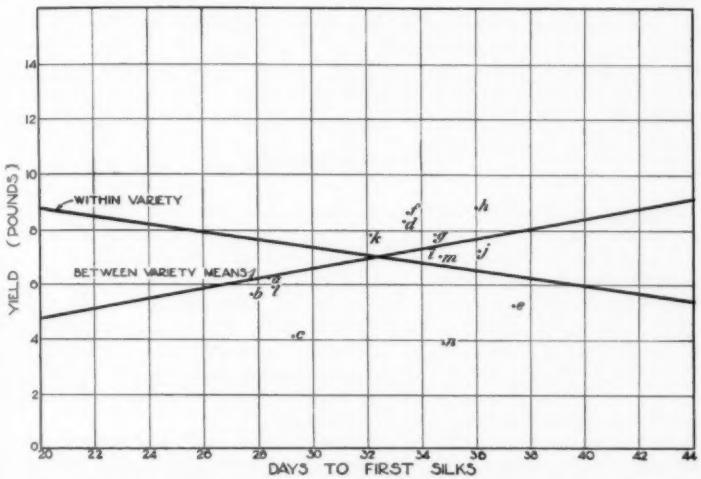

FIGURE 2.—Regressions of yield on days to first silks, within variety and between variety means, for 14 varieties of maize: *a*, Four County White; *b*, Silver King; *c*, U. S. Selection 133; *d*, Iodent; *e*, U. S. Selection 204; *f*, Lancaster Surecrop; *g*, Black Yellow Dent; *h*, Proudfit Yellow Dent; *i*, Krizer Bros. Yellow Dent; *j*, McCulloch Yellow Dent; *k*, Osterland Yellow Dent; *l*, Clark Yellow Dent; *m*, Walden Dent; *n*, Argentine Flint.

errors of estimate are as conspicuous as the trend. The same tenuity of relation is exhibited again in table 5. The mean square error of estimate between means of varieties is almost as large as the mean square for yield, showing that little of the variability of variety yields is associated with differences in earliness. The final source of evidence is the test of the significance of the mean square error of estimate. Since

$$F = 21.16/3.50 = 6.05$$

far surpasses the 1 percent level (*6, table XXXV*), the mean variety yields deviate from their regression much more than can be explained by sampling variation. Under such circumstances, one cannot lightly assume that the regression of means corresponds to a biological entity. However, as indicated before, a positive regression has a reasonable interpretation in this experiment, and receives some support in the remaining effect to be discussed below.

It is of interest to observe the contrast between the total correlation coefficient of table 4, 0.0093, and that within variety, −0.2419. The latter is the correct average of the 14 intravariety coefficients. Now if all the data of table 1 had been thrown together, without consideration of the existing differences among the variety means, the significant within-variety correlation between yield and earliness would have been undetected, being automatically averaged with the between-variety coefficient, 0.3591, to make the total nonsignificant coefficient, 0.0093. This emphasizes the importance of isolating and studying the statistics of homogeneous groups in a large experiment. If it is desirable, these groups may be merged later, but with full knowledge of the homogeneity or heterogeneity of the entire mass of data.

In the right-hand part of table 5 there remains a degree of freedom which has not been discussed. It is attributed to the difference between the two regressions within and between varieties—that is, between the slopes of the two regression lines of figure 2. The mean square, 65.00, furnishes the test ot significance of this difference:

$$F=65.\,00/3.\,50=18.6,$$

a value far beyond the 1-percent level. It has been demonstrated before that one of these regressions differs significantly from zero while the other does not. It is now proved that the difference between them is highly significant. While the interpretation is clouded somewhat by the lack of a clear-cut linear relation among the variety means, discussed above, the indication is that the results of the genetic factors influencing the relation between earliness and yield in the varieties examined may be distinguished from those predominantly physiological and environmental governing this relation among the inbred lines of the varieties.

Some experiments are planned with the knowledge that neither the individual regressions nor the regression of means will differ significantly from the pooled within-group regression. In the earliness-yield problem which has been discussed, the former condition was met, but not the latter. When field-plot experiments are laid out in the randomized block or Latin square designs, there is every reason to expect that the regressions will be uniform. In such data, most of the computations described above may be omitted. The only ones necessary are those extracted from table 4 and set out in table 6. Merely the total regression and that within variety are used, with consequent reductions in the degree of freedom for errors of estimate. The sum of squares of errors of estimate of the variety means is got by subtraction. These errors are measured from the average within-variety regression. Since the regression of means is not used in the computation, no reduction is suffered in degrees of freedom for the errors of estimate of these means. The appropriate test of significance is

$$F=24.\,5/3.48=7.\,1,$$

highly significant as in table 5. If this particular information is the extent of that required, the foregoing computations may be still further abbreviated in the manner described by Fisher (*3, p. 257*) as well as by Snedecor (*7*) and Cox and Snedecor (*2*).

TABLE 6.—*Errors of estimate of variety means measured from the pooled "within-variety" regression*

[Data taken from table 4]

Source of variation	Degrees of freedom	Sums of squares and products			Errors of estimate		
		Time to silks Sx^2	Products Sxy	Yield Sy^2	Sum of squares $Sy^2-[(Sxy)^2/Sx^2]$	Degrees of freedom	Mean square
Total	141	2,489.47	12.83	761.01	760.94	140	
Pooled within variety	128	1,359.28	−193.28	469.54	442.05	127	3.48
Between-variety means	13				318.89	13	24.5

SUMMARY

There is presented an analysis of the covariance between yield and time to first silks among inbred lines and 14 varieties of maize.

The 14 variety regressions of yield on time to first silks do not differ significantly among themselves, presenting no evidence against the hypothesis that there is a common regression in all the inbred lines.

The average within-variety regression differs significantly from that between-variety means, showing that variety mean yields do not respond to variety earliness in the same manner as the yields of inbred lines respond to their earliness. The variety means depart significantly from their regression, emphasizing this finding. There is included a convenient method for averaging the correlation coefficients of the 14 varieties.

An easy test appropriate for field plot trials is given.

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A CRITERION FOR TESTING THE ACCURACY OF TRAP-NEST RECORDS

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INTRODUCTION

A test of the accuracy of monthly trap-nest records constitutes an important phase of poultry record-of-performance inspection work. A recent discussion of inspection work in Ohio, presented by Amiet [2], gives some idea of the kinds of tests which are being used by the more critical inspectors at the present time. The utility of such tests is limited by the fact that adequate information relating to the variability to be expected under normal conditions is not available.

It is the purpose of the present paper to offer a test of the accuracy of monthly trap-nest records which is free from this defect. The test is intended to supplement, rather than to supplant, those already in use. It is designed to determine whether or not the number of eggs laid by a given flock of birds on inspection days differs significantly from the production to be anticipated from a consideration of the production reported by the flock owner for other days.

MATHEMATICAL THEORY

Suppose that a flock of birds is trap-nested by the inspector one or more days each month for several months. Let s represent the number of months. Let k_i denote the number of days in the inspection period for the ith month, and let n_i denote the number of birds in the flock during that inspection period. Let x_i denote the number of eggs produced during the inspection period. If p_i represents the probability that a bird will produce an egg on an inspection day, the expected number of eggs for the entire inspection period in that month is equal to $k_i n_i p_i$.

If x_i represents the number of eggs actually produced during the inspection period and $k_i n_i p_i$ represents the number expected, the probability of obtaining a number of eggs between x_i and x_i+dx_i is given approximately by the equation:

$$dF_{x_i}=\frac{1}{[2\pi k_i n_i p_i(1-p_i)]^{\frac{1}{2}}}\, e^{-\frac{1}{2}\frac{(x_i-k_i n_i p_i)^2}{k_i n_i p_i(1-p_i)}}\, dx_i \qquad (1)$$

Equation (1) may be written in the form:

$$dF_{t_i}=\frac{1}{(2\pi)^{\frac{1}{2}}}\, e^{-\frac{1}{2}t_i^2} dt_i \qquad (2)$$

in which:

$$t_i=\frac{x_i-k_i n_i p_i}{[k_i n_i p_i(1-p_i)]^{\frac{1}{2}}} \qquad (3)$$

[1] Received for publication Dec. 11, 1936; issued May, 1937.

[2] AMIET, E. R. METHODS EMPLOYED IN DOING U. S. R. O. P. WORK IN OHIO. U. S. Dept. Agr., Bur. Anim. Indus., Conference on the National Poultry Improvement Plan in Chicago, Ill. pp. 79-88, illus. Washington. 1936. [Mimeographed.]

The probability of obtaining an observed combination of numbers of eggs from the various inspection periods such as a number between x_1 and x_1+dx_1 the first month and between x_2 and x_2+dx_2 the second month, is given by the equation:

$$dF_{t_1,\ t_2,\ \cdots\ t_s} = \frac{1}{(2\pi)^{\frac{1}{2}s}} e^{-\frac{1}{2}(t_1^2+t_2^2+\cdots+t_s^2)} dt_1 dt_2 \cdots dt_s \qquad (4)$$

If the quantity $(t_1^2+t_2^2+\cdots+t_s^2)$ in equation (4) is denoted by χ^2, the equation may be written in the form:

$$dF\chi^2 = \frac{1}{\Gamma(\frac{1}{2}s)\,2^{\frac{1}{2}s}} e^{-\frac{1}{2}\chi^2} (\chi^2)^{\frac{1}{2}(s-2)} d(\chi^2) \qquad (5)$$

Equation (5) is of particular interest because values of its integral have been tabulated by biometricians. It is, in fact, identical with the equation giving the sampling distribution of the familiar criterion of goodness of fit, as applied to frequency distributions, for s degrees of freedom.

The quantity, χ^2, defined by the relation:

$$\chi^2 = \sum_{i=1}^{s} \frac{(x_i - k_i n_i p_i)^2}{k_i n_i p_i (1-p_i)} \qquad (6)$$

is, therefore, distributed according to a well-known law under normal conditions. However, before its numerical value can be calculated for a given set of data, it is necessary to obtain estimates of the numerical values of the p_i. Under normal conditions, a satisfactory estimate of each of the p_i is given by the egg production per bird per day over a period including at least several days before and after inspection. The data obtained during the inspection period should preferably be omitted in obtaining this estimate. Although such data may properly be included if the records are accurate, it is desirable to omit them in obtaining a test of the accuracy of the records.

To test the accuracy of a given set of trap-nest records, all that is required is to calculate the numerical value of χ^2 for the given set of data according to equation (6) and to make use of the tables of values of χ^2 given by Fisher [3] or Pearson [4] to determine whether or not that value is excessively large for s degrees of freedom. An excessively large value of χ^2 would indicate that the given set of trap-nest records should be regarded with suspicion.

EXPERIMENTAL VERIFICATION OF THE THEORY

In order to verify the hypothesis that the quantity χ^2, defined by equation (6), is distributed in the same manner as the well-known criterion of goodness of fit under normal conditions, the author calculated 100 such values from data obtained for 100 lots of birds used in various nutrition investigations at the National Agricultural Research Center, Beltsville, Md.[5] The fifteenth day of the month for each of the 3 months, March, April, and May, was assumed to be an inspection day. There were from 10 to 48 birds in any one lot on an

[3] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, 319 pp., illus. Edinburgh and London. 1934.

[4] PEARSON, K. TABLES FOR STATISTICIANS AND BIOMETRICIANS. Ed. 2, Pt. I, 226 pp., illus. Cambridge. 1924.

[5] Kate W. Robey of the Animal Husbandry Division assisted in this phase of the work.

assumed inspection day, and the number of eggs produced on this day ranged from 1 to 32. In every lot of birds, the egg production per bird per day for an entire month was used as the value of p_i for that month.

The frequency distribution of the 100 values of χ^2 obtained is given in table 1. The corresponding theoretical distribution, derived from tables of the criterion of goodness of fit for 3 degrees of freedom, is included for comparison.

TABLE 1.—*Observed and theoretical distributions of 100 values of χ^2, each calculated for 3 monthly inspection days from normal trap-nest records*

Value of χ^2	Observed frequency	Theoretical frequency	Value of χ^2	Observed frequency	Theoretical frequency	Value of χ^2	Observed frequency	Theoretical frequency
0.0–0.9	25	19.9	5.0–5.9	11	6.0	10.0 and over	2	1.9
1.0–1.9	16	22.9	6.0–6.9	1	4.0			
2.0–2.9	15	18.0	7.0–7.9	4	2.6	Total	100	100.0
3.0–3.9	14	13.1	8.0–8.9	5	1.7			
4.0–4.9	7	8.9	9.0–9.9	0	1.0			

The agreement between the observed and theoretical distributions is very good. The degree of discrepancy between observation and theory in the table could arise by chance about once in five trials.

From the results obtained it appears that the proposed method of testing the accuracy of trap-nest records is sound. However, the author wishes to call particular attention to the need for caution in obtaining estimates of the values of the p_i used in equation (6). The egg production per bird per day serves as a valid estimate of the probability, p_i, only when this quantity may justifiably be assumed to remain constant, except for sampling fluctuations, over the period of time used in its computation. In the data used for the experimental work described, this condition was satisfied. If there had been some logical reason for believing that the egg production per bird per day did not remain fairly constant during an entire month for a given lot of birds, this quantity should have been calculated from data obtained during a shorter period just before or after the inspection day for that month.

EXAMPLE OF APPLICATION OF METHOD

In spite of the somewhat involved mathematics employed in the derivation of the present test, its practical application is simple. In the following example are presented the details of the application of the test to one of the sets of trap-nest records used in the preceding experimental verification of the theory.

TABLE 2.—*Computation of χ^2 for a set of trap-nest records involving three inspection periods*

Month	x_i	k_i	n_i	p_i	$k_i n_i p_i$	$k_i n_i p_i(1-p_i)$	$\frac{(x_i-k_i n_i p_i)^2}{k_i n_i p_i(1-p_i)}$
March	16	1	22	0.657	14.45	4.96	0.48
April	12	1	22	.698	15.36	4.64	2.43
May	12	1	22	.638	14.04	5.08	.82
Value of χ^2							3.73

Table 2 presents the steps in the computation of χ^2 for such a set of records. The x_t represent the numbers of eggs produced by the given lot of birds during the monthly inspection periods. Since the length of each inspection period was only 1 day, the value of each of the k_t is unity. The n_t represent the numbers of birds in the lot which were alive during the inspection periods, and the p_t represent the egg production per bird per day for the 3 months. The remaining entries in the table require no explanation. It is evident that the process of computing the value of χ^2 represents a comparison of the observed egg production, x_t, during each inspection period with the corresponding expected production, $k_t n_t p_t$.

The value of χ^2 for this set of records is 3.73. Since there were three monthly inspection periods, a table of values of χ^2 must be examined to determine whether or not this value is excessively large for three degrees of freedom. Such an examination reveals that a value of 3.73 or larger is likely to arise by chance about 29 times in 100 trials. Therefore, the value, 3.73, is not excessively large and the given set of records may be assumed to be accurate.

In the practical application of the test, it is impossible to make a definite statement in regard to the maximum value of χ^2 which can be accepted as arising by chance. All that can be done is to set some arbitrary limit, such that the probability of exceeding it by chance is very small, and then subject to a very critical examination any records yielding a value of χ^2 in excess of this limit.

The following tabulation presents values of χ^2 which are likely to be equaled or exceeded by chance only once in 100 trials, for normal trap-nest records involving various numbers of inspection periods. These values were taken directly from the table of values of χ^2 given by Fisher [6] and may be regarded as the largest values likely to arise by chance in the case of normal trap-nest records.

Number of inspection periods:	*Value of* χ^2	Number of inspection periods—Continued.	*Value of* χ^2
1	6. 635	7	18. 475
2	9. 210	8	20. 090
3	11. 341	9	21. 666
4	13. 277	10	23. 209
5	15. 086	11	24. 725
6	16. 812	12	26. 217

SUMMARY

Existing methods of testing the accuracy of trap-nest records in poultry record-of-performance inspection work are not fully satisfactory because of a lack of information regarding the amount of variability to be expected under normal conditions. A new test, which may be employed to supplement those in use at the present time, is free from this defect. It involves the calculation of a statistic which, under normal conditions, is distributed in the same manner as the well-known χ^2 criterion of goodness of fit as applied to frequency distributions. The number of monthly inspection periods corresponds to the number of degrees of freedom which specifies the form of the latter distribution.

[6] FISHER, R. A. See footnote 3.

EFFECT OF INCUBATION TEMPERATURE ON TIME OF DEATH OF CHICK EMBRYO AND RELATIONSHIP OF ENERGY METABOLISM TO MORTALITY[1]

By EMMA M. PRINGLE, *assistant scientific aide*, and H. G. BAROTT, *poultry technologist, Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Poultrymen have long known that there are certain periods during incubation when the mortality of developing chick embryos far exceeds the average. The present investigation was undertaken to obtain information about these critical periods which cause great losses to the poultry industry. The effect of temperature on the time of death of the embryo and the relationship between energy metabolism and mortality during incubation were studied.

PROCEDURE

The research here reported was conducted at the National Agricultural Research Center, Beltsville, Md., from 1932 to 1935. More than 20 complete experiments were made, with 878 fertile eggs incubated at 102° F. and 791 eggs at 99°.

The eggs used were obtained from a selected flock of Single Comb White Leghorn hens which were trap-nested. The hens were fed a standard, well-balanced diet calculated to produce eggs of high fertility and hatchability. The eggs were collected every 3 or 4 hours, at which time they were marked with the hen number and date. No eggs incubated were more than 2 weeks old, and most of them were less than 1 week old. Before being placed in the calorimeter they were carefully inspected for cracks and other defects, and only those of normal shape and size were used. The eggs were turned once every 6 hours to the fifteenth day. They were candled on the seventh and fifteenth days by an experienced operator, at which times all dead embryos were removed. After the second candling the eggs were put in hatching boxes and replaced in the calorimeter, which was not opened again until the time of hatch.

The eggs were incubated in a horizontal position in two respiration calorimeters. The apparatus and methods of use have been described by Barott.[2] All experiments were made under rigidly controlled conditions. Measurements of the complete energy metabolism of the developing embryo were made every 8 hours. The time of death of an individual embryo could be detected by a change in the rate of heat elimination, thus fixing the time to the nearest 8 hours. The conditions inside the calorimeter other than temperature were: Relative humidity, 60 to 70 percent; oxygen, 21 percent; and carbon dioxide, below 0.5 percent.

[1] Received for publication Dec. 31, 1936; issued May, 1937.

[2] BAROTT, H. G. EFFECT OF TEMPERATURE, HUMIDITY, AND OTHER FACTORS ON HATCH OF HENS' EGGS AND ON ENERGY METABOLISM OF CHICK EMBRYOS. U. S. Dept. Agr. Tech. Bull. 553, 46 pp., illus. 1937.

RESULTS

Figure 1 shows the daily mortality, at 99° and 102° F., expressed as a percentage of the total mortality during incubation. The usual well-defined, critical period was found with the peak on the third day at both temperatures. From the fourth to the tenth day the mortality

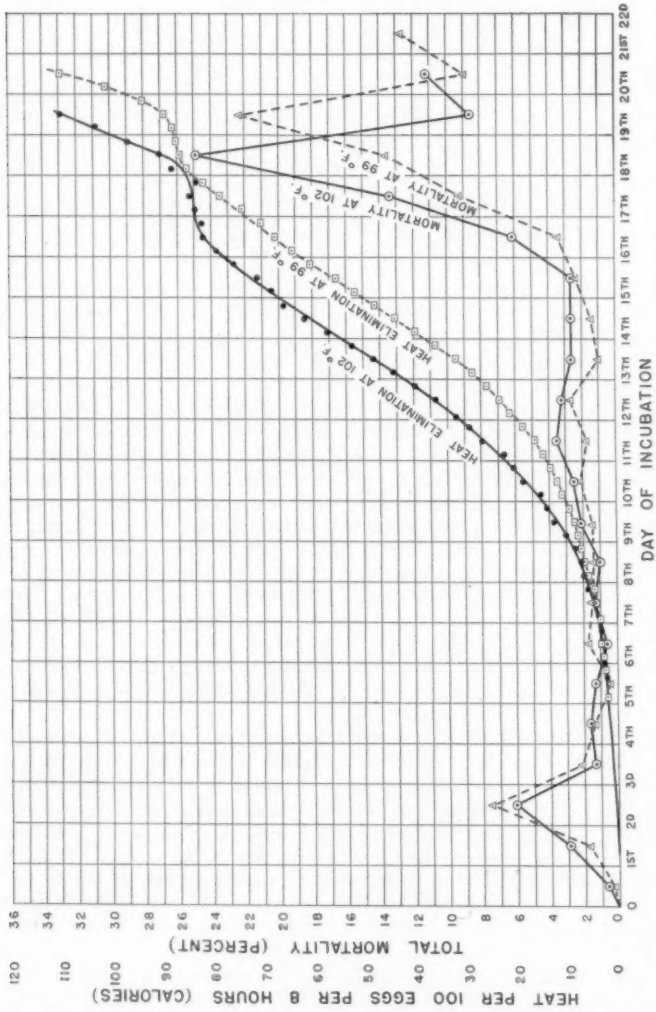
MORTALITY AT 99 °F.
MORTALITY AT 102 °F.
HEAT ELIMINATION AT 99 °F.
HEAT ELIMINATION AT 102 °F.
DAY OF INCUBATION
1ST 2D 3D 4TH 5TH 6TH 7TH 8TH 9TH 10TH 11TH 12TH 13TH 14TH 15TH 16TH 17TH 18TH 19TH 20TH 21ST 22D
TOTAL MORTALITY (PERCENT)
HEAT PER 100 EGGS PER 8 HOURS (CALORIES)

FIGURE 1.—Effect of incubation temperature on heat elimination and mortality of chick embryo. (The last point on each mortality curve represents embryos which were alive but not hatched and which died ultimately.)

was nearly constant at a daily average of 1.5 percent. The average daily mortality between the tenth and sixteenth days was somewhat higher at 102° than at 99°. At both temperatures it was nearly constant over this period. The typical second period of high mor-

tality occurred with the peak on the nineteenth day at 102° and on the twentieth day at 99°. Barott [3] has shown that the eggs hatch approximately 33 hours earlier at 102° than at 99°. The fact that the peak in mortality was delayed at 99° by practically the same time indicates that it is closely associated with the stage of development of the chick embryo.

Table 1 gives the percentage of distribution of the mortality in the first and second critical periods at both temperatures.

TABLE 1.—*Distribution of mortality in the two critical periods of incubation*

Temperature of incubation (° F.)	Percentage of total mortality occurring during—		
	First critical period	Second critical period	Both periods
	Percent	*Percent*	*Percent*
99	11.3	53.4	64.7
102	10.3	53.0	63.3

The results at both temperatures show almost two-thirds of the deaths occurring in the two critical periods, with approximately one-half of all mortality during the second period. However, at 99° the hatch was 70 percent of the fertile eggs set, whereas at 102° it was 60 percent. Although the lower temperature results in a larger hatch and less mortality, table 1 shows that the distribution of dead embryos is nearly the same at both temperatures.

Figure 1 also shows the heat elimination of the developing embryo at the two temperatures. The rate of increase in metabolism for the first 7 days is uniform. During the seventh to the twelfth days at 102° and the seventh to fourteenth days at 99° there is a greatly accelerated and constantly increasing rate. Between the twelfth and sixteenth days at 102° and the fourteenth and eighteenth days at 99° the rate of acceleration becomes uniform again but greatly increased over any previous rate. On the seventeenth day at 102° and on the nineteenth day at 99° there is an abrupt change in energy metabolism, the acceleration practically ceases, and the heat production remains nearly constant for a period of approximately 1 day. After this, it increases more rapidly than at any time previously. The peak of the second period of mortality occurs at the end of the period when the heat production is constant. This indicates a close association between metabolism and mortality.

DISCUSSION

The first day of incubation is primarily a period of cell differentiation, with the formation of the rudimentary chick taking place during the second and third days. During this time the heart begins to beat and the formation of the primary division of the brain and central nervous system occurs. The chick is well defined by the fourth day. The high mortality observed during this early period is probably due to inherited factors, or environmental factors previous

[3] BAROTT, H. G. See footnote 2.

to incubation. Most embryos at least begin growth, but those not having the potentialities for complete development of the complicated circulatory and nervous system die during this time even with optimum conditions of incubation.

The embryos develop at a uniformly increasing rate from the fourth to the tenth day, during which time the mortality is very low. The energy used by the embryo to the fourth day is principally carbohydrate. From the fourth to the tenth day there is a high protein metabolism, whereas during the latter part of incubation fat is the principal source of energy. The increase in mortality about the tenth day occurs after a period of intense protein metabolism and may be associated with the concentration of protein derivatives in the amniotic fluid.

The great increase in mortality from the seventeenth to the twentieth day may be the result of very important physiological changes which occur during that period. The respiratory system undergoes a complete change, the beak of the embryonic chick breaks into the air cell of the egg, and pulmonary respiration begins. The yolk is absorbed into the body cavity by a more or less complex mechanism involving considerable muscular contraction. In fact, such profound and complex changes might very well be fatal to any embryo with less than normal vigor. This may explain why embryos die at this time, but it does not answer the question why the vitality of the embryo is not sufficient for it to survive these difficult changes. Probably the answer to this lies in the metabolism of the embryo during the previous 2 weeks of incubation.

CONCLUSIONS

From results obtained by incubating hens' eggs in a respiration calorimeter which permitted rigid control of all factors governing incubation, the following conclusions are drawn:

There are two critical periods of mortality at incubation temperatures of 99° and 102° F. in which approximately two-thirds of all mortality occurs.

The first period occurs at the same time at either temperature, the peak of mortality being on the third day in the experiments conducted. The second period occurs shortly before the normal time of hatching. The peak of mortality was on the twentieth day for eggs incubated at 99° and on the nineteenth day for those at 102°; at both temperatures high mortality occurred immediately after an abrupt change in energy metabolism. The results indicate a close relationship between the stage of development of the chick embryo and mortality.

A comparison of the energy metabolism and mortality curves of the growing embryo shows an association between rate of change in energy metabolism and mortality.

RELATIVE TOXICITY OF THE CRESOLS AS DEMONSTRATED BY TESTS WITH CARASSIUS AURATUS[1]

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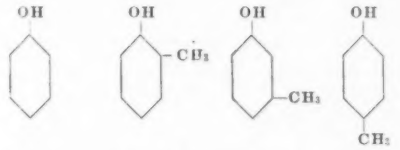
INTRODUCTION

The introduction of the methyl group into the nucleus of phenol may affect the toxicity differently according to the relative positions of the hydroxyl and the methyl groups. The relative toxicity of the three isomers formed, however, has not always been reported to be the same, even against the same test animal, by different investigators. The writer believes that this may be due largely to differences in the definitions of toxicity and to inadequate methods of obtaining the data in support of those definitions. In an effort to place a toxicological study of these substances on a quantitative basis, a series of tests has been performed with them over a range of concentrations sufficiently wide to permit such a comparison.

The structural relationship of the compounds is shown by their formulas, as follows:



phenol *o*-cresol *m*-cresol *p*-cresol

Freshly redistilled, colorless samples were used. The phenol was chemically pure. The original samples of the cresols contained less than 2 percent of impurities, which in each case consisted of the other two isomers. Since the compounds do not differ greatly in toxicity, the samples were of a sufficiently high degree of purity for the purposes of this study.

As in previous studies (*5*, *6*, *7*, *8*, *9*),[2] the goldfish (*Carassius auratus*) was used as the test animal, and for comparative purposes tests were also made with rotenone. Inasmuch as the toxic action of phenol (*9*) has been found to be quite different from that of rotenone and the action of the cresols would presumably be similar to that of phenol, a number of tests were also made with this compound on the same lot of fish.

EXPERIMENTAL PROCEDURE

The method of making the toxicity tests was essentially the same as that previously described (*4*). Goldfish of a single lot were used, and a constant temperature of 27°±0.2° C. was maintained. Since fish of the small grade were unobtainable at this time, fish of larger size, and therefore of greater resistance, had to be used. They weighed approximately 6.0 to 7.5 g each.

[1] Received for publication Nov. 16, 1936; issued May 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 477.

The phenolic test solutions were made to the desired concentrations with aliquots from aqueous stock solutions, a 5-percent solution of phenol and 2-percent solutions of the cresols because of their lesser solubility. Acetone was used as usual in making the solutions of rotenone.

The increase in hydrogen-ion concentration caused by the addition of the phenols was not great enough to be considered a disturbing factor.

RESULTS

The type of toxic action of the cresols was found to be similar to that of phenol (*9*).

Since skew frequency distributions of the observed survival times were obtained in both cases, the results will be treated in the same way as in the previous paper (*9*); that is, the geometric mean rather than the arithmetic mean will be the form of average selected as defining the position of each distribution.

TABLE 1.—*Toxicity of rotenone, phenol, and the cresols to goldfish at 27.0° ±0.2° C.*

Compound and concentration (milligrams per liter)	Fishes used	Mean length of fishes	Mean weight of fishes [1]	Mean survival time [2]			1,000÷ geometric mean survival time
				Arithmetic	Geometric		
Rotenone:	*Number*	*Millimeters*	*Grams*	*Minutes*	*Minutes*		
1.00	12	64	7.3	132± 5	129	×/÷ 1.03	7.75
.50	12	62	6.9	152± 6	150	×/÷ 1.04	6.67
.30	12	62	6.9	204±17	188	×/÷ 1.08	5.32
.20	9	64	7.4	216±13	210	×/÷ 1.06	4.76
.15	12	62	6.8	251±22	230	×/÷ 1.10	4.35
.10	12	62	6.9	395±46	341	×/÷ 1.12	2.93
.086	14	65	7.6	520±28	490	×/÷ 1.07	2.04
.075	11	62	6.8	737±59	702	×/÷ 1.08	1.42
Phenol:							
414	8	63	7.1	29± 3	27	×/÷ 1.10	37.0
349	9	61	6.6	43± 9	31	×/÷ 1.16	32.3
299	12	62	6.8	48± 8	35	×/÷ 1.19	28.6
249	11	61	6.6	106±24	60	×/÷ 1.30	16.7
208	13	61	6.6	131±30	76	×/÷ 1.24	13.2
100	11	59	6.0	424±66	310	×/÷ 1.21	3.23
o-cresol:							
339	7	64	7.4	27± 1	27	×/÷ 1.01	37.0
255	12	63	7.1	42± 3	36	×/÷ 1.04	27.8
171	10	61	6.6	72± 3	70	×/÷ 1.05	14.3
161	29	62	6.8	90± 6	84	×/÷ 1.08	11.9
150	12	65	7.5	133±14	119	×/÷ 1.10	8.40
137	10	61	6.5	261±37	217	×/÷ 1.17	4.61
103	10	65	7.6	484±42	453	×/÷ 1.10	2.21
69	10	59	6.0	537±42	500	×/÷ 1.10	2.00
m-cresol:							
422	9	63	7.1	30± 2	29	×/÷ 1.07	34.5
284	10	62	6.8	61± 3	59	×/÷ 1.05	16.9
227	12	62	6.7	82± 6	76	×/÷ 1.09	13.2
171	12	62	6.8	204±23	169	×/÷ 1.14	5.92
143	12	65	7.5	223±24	185	×/÷ 1.15	5.41
129	12	63	7.0	359±49	301	×/÷ 1.13	3.32
115	11	62	6.9	418±56	329	×/÷ 1.20	3.04
86	11	65	7.5	499±73	408	×/÷ 1.14	2.45
p-cresol:							
280	13	61	6.5	27± 1	27	×/÷ 1.01	37.0
169	10	62	6.9	34± 3	33	×/÷ 1.08	30.3
155	14	60	6.3	57± 4	53	×/÷ 1.08	18.9
141	12	62	6.8	128±42	49	×/÷ 1.17	20.4
127	9	61	6.5	88±18	66	×/÷ 1.13	15.2
113	12	63	7.1	226±66	96	×/÷ 1.27	10.4
85	9	61	6.5	617±168	283	×/÷ 1.48	3.53

[1] Estimated from length, which measurement excludes the tail.

[2] The limits of error indicated are probable errors of the means. In the case of the arithmetic means they are differences from the means; in the case of the geometric means they are ratios to the means.

The toxicological data are given in table 1. The survival-time curves and the velocity-of-fatality curves which are plotted from these

data are given in figures 1 and 2, respectively. The curves for rotenone are not shown because they require a much more expanded scale. In a few cases where intermediate points were not determined experimentally because of the small differences in survival time, the intervals between successive concentrations appear to be relatively large. Since the values for survival time are small, the apparent scattering of the determined points is exaggerated in the plotting of the reciprocals. To permit the more ready location of these portions of the velocity-of-fatality curves in these cases, especially in the neighborhood of a critical point to be discussed later, intermediate points were obtained from interpolated points on the survival-time curves. This procedure is justified by the fact that, with the use of geometric means, the reciprocal of the mean survival time is equal to the mean of the

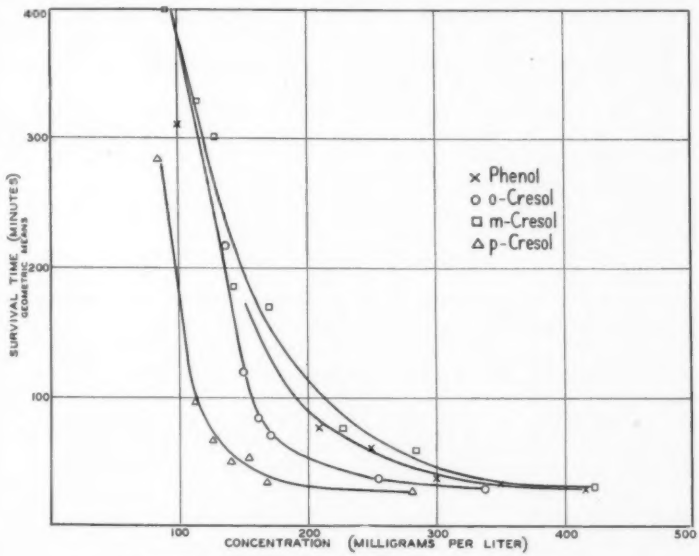


FIGURE 1.—Survival-time curves for phenol and the cresols.

reciprocals, and therefore the two types of curves actually have a reciprocal relationship, which is not true when arithmetic means are used.

DISCUSSION OF DATA

It is apparent from figure 2 that cresols resemble phenol in their general mode of progression of toxic action with increase in concentration. They all give the distinctly sigmoid curvature of the velocity-of-fatality curve which has characterized paralytic action in previous studies. They differ in the main in the acceleration of this action. This difference is most easily recognized in the range of greatest acceleration. Within this range the curve passes through the point of inflection, and since it approaches a straight line it is represented by a straight line in figure 2. The most efficient action is in the upper limit of this range, where the velocity of fatality is highest before acceleration is appreciably retarded.

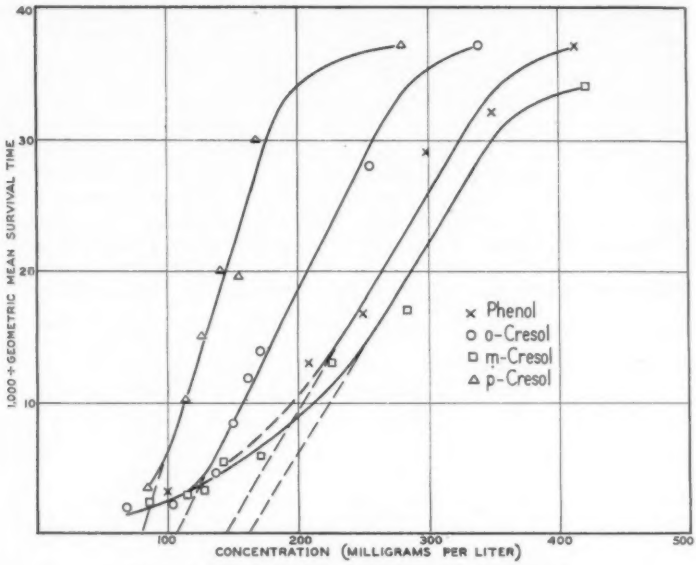


FIGURE 2.—Velocity-of-fatality curves for phenol and the cresols.

A measure of toxicity in this range is the criterion proposed by the author (*8*), the minimum product of concentration and survival time. This value was calculated directly from the survival-time curve. It may, however, be determined geometrically from the velocity-of-fatality curve by drawing the maximum tangent to the curve from the origin. The slope of this line, expressed in the units of the two variables, will give the reciprocal of the minimum product. This procedure is made possible by the fact that the maximum tangent to the velocity-of-fatality curve through the origin corresponds to the equilateral hyperbola tangent to the survival-time curve at the critical point.

The data for comparison by this measure are given in table 2, the minimum product of concentration and time being designated by $c_m t_m$. Since toxicity varies inversely with this value, its reciprocal is also given. Its approximate coordinates, c_m and t_m, are given so that the region of the curve fulfilling this condition can be located readily.

TABLE 2.—*Relative toxicity of rotenone, phenol, and the cresols at 27° C.*

Compound	c_m	t_m	$c_m t_m$	Toxicity, $\frac{1}{c_m t_m}$	Relative toxicity according to $c_m t_m$
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	
Rotenone	0.120	277	0.0332	30.1	1.0
Phenol	350	31	10.9	.0917	.0030
o-cresol	290	31	9.00	.111	.0037
m-cresol	360	32	11.5	.0870	.0029
p-cresol	190	30	5.70	.175	.0058

From these data it is seen that according to this criterion phenol is only 0.0030 as toxic as rotenone, a ratio that closely approximates that (0.0032) found previously (*9*) in tests with smaller fish. *m*-cresol, the least toxic of the cresols, is slightly less toxic than phenol. *o*-cresol is one-fifth more toxic than phenol. *p*-cresol, the most toxic of the cresols, is nearly twice as toxic as phenol.

COMPARISON OF DATA OBTAINED BY VARIOUS METHODS

Since the quantitative comparison of toxicity is so intimately associated with the method of defining relative toxicity, the data obtained were studied in relation to the several methods that have been proposed for use in such a study with respect to the two variables—concentration and time. The data for these criteria are given in tables 3 and 4.

TABLE 3.—*Data used in determining the relative toxicity of the cresols, phenol, and rotenone*

Compound	c_0	t_0	$\tan\theta$	$\sqrt{\frac{\tan\theta}{c_0}}$	$\sqrt{\frac{\tan\theta}{c_0 t_0}}$	$\frac{1}{c_m t_m}$	$\frac{1}{(c_m-c_0)(t_m-t_0)}$
	Milligrams per liter	*Minutes*	*Liters per gram per minute*		*Liters per gram per minute*	*Liters per gram per minute*	*Liters per gram per minute*
m-cresol	100	15	0.157	0.994	0.256	0.0870	0.294
Phenol	145	15	.167	1.07	.276	.0917	.305
o-cresol	107	15	.200	1.35	.353	.111	.341
p-cresol	80	15	.308	1.96	.506	.175	.606
Rotenone	.053	120	58.2	1,050	95.7	30.1	95.2

TABLE 4.—*Relative toxicity of the cresols, phenol, and rotenone as determined by different methods, expressed as ratios to the toxicity of m-cresol*

Compound	$\tan\theta$	$\sqrt{\frac{\tan\theta}{c_0}}$	$\sqrt{\frac{\tan\theta}{c_0 t_0}}$	$\frac{1}{c_m t_m}$	$\frac{1}{(c_m-c_0)(t_m-t_0)}$	$\frac{1}{c_0}$	$\frac{1}{c_m}$
m-cresol	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phenol	1.06	1.08	1.08	1.05	1.04	1.10	1.03
o-cresol	1.27	1.38	1.38	1.28	1.16	1.50	1.24
p-cresol	1.96	1.98	1.98	2.01	2.06	2.00	1.89
Rotenone	371	1,060	374	346	327	3,020	3,000

The first criterion (*8*, *15*) is the slope ($\tan\theta$) of the theoretical velocity-of-fatality curve, which is the straight line approximating that portion of the velocity-of-fatality curve corresponding to the greatest rate of increase in the velocity of fatality with increase in concentration. With the phenolic compounds it has a more definite value than with the rotenone compounds, because the line, which must pass through the point of inflection, is drawn with more ease. The intersection of this line, when prolonged, with the X-axis is designated c_0, and the second criterion, $\sqrt{\frac{\tan\theta}{c_0}}$, proposed by Powers (*14*), is based on these two parameters. The third criterion, $\sqrt{\frac{\tan\theta}{c_0 t_0}}$, is the writer's suggested modification (*8*) of Powers' formula. It introduces a third parameter, t_0, the survival time corresponding to the

region of constant velocity. Its value was obtained by an extrapolative procedure such as was used to obtain the theoretical threshold values, that is, by plotting the reciprocal of the concentration against survival time and using the points corresponding to the minimum *ct* products as approximating the upper limits of the straight lines. It must be emphasized that this is not the minimum time in which fish may be killed in solutions of these substances, for in the range of comparatively high concentrations there may again occur a region of acceleration (*7*, *9*, *15*), but this is probably due to a different type of toxic action. It is, however, a value that is an approximation of the condition of constant velocity occurring over the range of concentrations just preceding this. The fourth criterion is the minimum product of concentration and time, $c_m t_m$. The fifth is a similar product obtained when the coordinates are referred to the two asymptotes as axes, being practically equal to $(c_m - c_0)(t_m - t_0)$.

It may be noted that, insofar as the phenolic compounds are concerned, the comparison of the values for $c_m t_m$, because of the closeness of the values of t_m, becomes practically a comparison of the values c_m; that is, a comparison of the concentrations at which the product of concentration and time is at a minimum. The actual values for $(c_m - c_0)(t_m - t_0)$ are approximate because of the approximate nature of t_0, but the relative values are not greatly affected by a variation in this value, since it appears to be nearly the same for all the compounds.

If we assume that a single magnitude can be used to express the relative toxicity with respect to concentration and time of compounds having such diverse toxic action as rotenone and phenol—that is, one of relatively high toxicity in the sense of concentration necessary to kill and yet requiring a relatively long time to act, and the other of low toxicity in the same sense and yet of rapid action—then which method is the best to use? Two of the methods mentioned consider only that portion of the curve where the acceleration of toxic action with increase in concentration is most rapid, and therefore disregard the two tolerance parameters as such, whereas the other three consider them. It has been previously suggested (*5*, *6*, *8*) that Powers' formula for expressing relative toxicity overemphasizes the effect of the threshold concentration in a comparison considering both concentration and time. In the present study also this formula operates more to the advantage of the substance with the low threshold than any of the other methods considered, despite the relatively small time parameters of the other substances. It may be noted from table 4 that with all the criteria except that of formula $\sqrt{\frac{\tan \theta}{c_0}}$ the values for rotenone do not differ greatly.

m-cresol was chosen as the standard of comparison to bring out at a glance the fact that, where the difference in the corresponding tolerance parameters of the various compounds is not great, all the methods give the same relative values. A graphical study of the meaning of the different criteria will show that this must be approximately so, since in this case they bear definite factorial relationship to one another.

If a comparison is to be made by the other type of measurement, in which the time factor is to be ignored—that is, in which there is no concern as to whether the substance is a quick- or a slow-acting poison—

it would be preferable to select a method using, rather than Powers' formula, a simple ratio of concentrations. For this purpose three concentrations may be considered—that at which the substance begins to become toxic, that at which it reaches its greatest toxic power, and that at which it reaches its greatest effectiveness or toxic saturation.

The first criterion would be comparable to the minimum-lethal-dose method of the pharmacologist. Since it is difficult to determine the actual thresholds of substances having such a slow initial acceleration as these phenolic compounds, it is possible to substitute the comparatively easily determined theoretical thresholds, c_0. For substances having velocity-of-fatality curves of the type shown for the phenols, the concentration corresponding to the point of inflection might be used as the criterion. This could not be used, however, for compounds having the rotenone type of curve, and it cannot be located so closely on the sigmoid curve as the concentration, c_m, corresponding to the minimum *ct* product. The last method may be disregarded as being relatively indefinite and having no simple, practical substitute.

The relative toxicity according to the first two criteria is given in the last two columns of table 4. It is obvious that both comparisons ignoring time favor the rotenone type of compound over the phenol type, as is to be expected.

COMPARISON OF RESULTS WITH THOSE OF OTHER INVESTIGATORS

Southgate (*18*) studied the toxicity of phenol and *p*-cresol as measured by the overturning time of yearling rainbow trout (*Salmo irideus*) from 2 to 4 inches in length at about 5° C. He stated that *p*-cresol was more toxic than phenol, a 0.0077-molar solution of the former having a toxic effect equivalent to a 0.011-molar solution of the latter. His data do not lend themselves to treatment similar to that in the present study, except for the comparison of theoretical threshold values. These values are approximately 7.8 mg per liter for phenol and 5.2 mg per liter for *p*-cresol, indicating that according to this measure *p*-cresol was about 1.5 times as toxic to these trout as phenol.

The values obtained for relative toxicity at the threshold have been compared with those reported by other workers against other test animals by the similar method, the comparison of the minimum lethal dose, as shown in table 5. The possibility of a general deduction is not implied by this comparison. Results may be compared properly only when relative toxicity is defined in the same manner, that is, when similar modes of toxic action are studied by the same method and compared according to the same criterion with the elimination of as many of the variable factors as possible. Nevertheless, attention may be called to the fact that only for the frog was phenol found by subcutaneous injection to be more toxic than all the cresols and in every case *p*-cresol was found to be the most toxic of the isomers. For the other test animals, despite the approximate nature of such results, the ratios are very near those found in this study of piscicidal activity, especially for the three cresols.

TABLE 5.—*Relative toxicity of phenol and the cresols according to the minimum lethal dose found by various workers, expressed as ratios to the toxicity of m-cresol*

Test animal [1]	*m*-cresol	Phenol	*o*-cresol	*p*-cresol	Worker
Rabbit	1.0	1.0	1.1	1.7	Meili (*12*).
Frog	1.0	2.5	1.3	1.7	Tollens (*21*).
Mouse	1.0	1.3	1.3	3.0	Do.
Cat	1.0	1.3	1.3	1.5	Do.
Rat	1.0	1.6	1.4	1.8	Binet (*1*).
Guinea pig	1.0	.7	.9	1.4	Do.
Dog	1.0		1.9	1.5	Gibbs and Hare (*10*).
Trout	1.0			1.5	Southgate (*18*).

[1] The substances were administered to the dog by intravenous injection, to the trout by addition to the water, and to the other animals by subcutaneous injection.

It must not be overlooked, however, that other workers, defining toxicity in an altogether different manner, have reported these phenolic compounds as having a different order of toxicity. This is especially so with respect to insecticidal (*13*, *19*, *20*) and bactericidal (*2*, *3*, *11*, *16*, *17*) action. Although the cresols are reported to be more toxic than phenol, *m*-cresol is usually placed as the most active of the isomers.

SUMMARY

A study was made of the toxicity of the three cresols with respect to concentration and survival time at 27° C., and the results were compared with each other and with those for phenol and rotenone. Goldfish of the same lot, weighing approximately 6.0 to 7.5 g each, were used as the test animals.

The type of toxic action of the cresols was found to be similar to that of phenol.

According to the minimum product of concentration and survival time, which measures toxicity at its range of most powerful action, *m*-cresol is slightly less toxic than phenol, *o*-cresol is one-fifth more toxic, and *p*-cresol is nearly twice as toxic. Phenol, according to this measure, is only 0.003 as toxic as rotenone.

The phenolic compounds do not differ much at the extremes of their ranges of toxic action. They differ mainly in the acceleration of this action with increase in concentration. Four methods of comparison in the range of greatest acceleration show that the compounds bear the following ratios of toxicity: *m*-cresol, 1.0; phenol, 1.1; *o*-cresol, 1.3; and *p*-cresol, 2.0. The same criteria show rotenone to be about 360 times as toxic as *m*-cresol.

If time is not considered in the measure of comparison, and the compounds are compared according to concentrations necessary to kill under certain conditions, the values for the relative toxicity of the phenolic compounds are changed but little, if at all, but rotenone is favored to such an extent that its relative toxicity becomes 3,000 times that of *m*-cresol.

The relative toxicity of these phenolic compounds according to the minimum lethal dose by subcutaneous injection found by other investigators for the rabbit, mouse, cat, rat, and guinea pig is close to that given above.

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